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(71) Applicant: BIOCHEMICAL MARKETING COR-PORATION [US/US]; 3506 N. Nottingham Street, Arlington, VA 22207 (US).

(72) Inventor: WELEBIR, Andrew, J.; 3506 N. Nottingham Street, Arlington, VA 22207 (US).

(74) Agent: STEWART, Raymond, C.; Birch, Stewart, Kolasch & Birch, 301 North Washington Street, P.O. Box 209, Falls Church, VA 22046-0209 (US).

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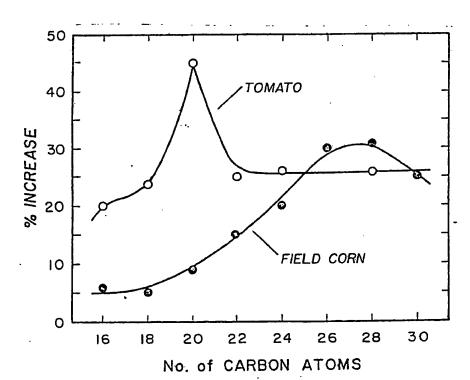
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(54) Title: PLANT GROWTH STIMULATORS COMPRISING METAL IONS AND LONG-CHAIN ALKYL CAR-BOXYLIC ACIDS AND SALTS AND DERIVATIVES THEREOF

#### (57) Abstract

Plant growth stimulator formulations comprising one or more longchain carboxylic acids having between 14 and 48 carbon atoms, and salts, esters, and derivatives thereof, and at least one metal ion having a valence of +2 to +3. The formulations of the invention are highly useful in stimulating the growth of plant life and yield of crops in the field when applied as foliar sprays, seed soaks, and soil drenches.



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# DESCRIPTION

PLANT GROWTH STIMULATORS COMPRISING METAL IONS AND LONG-CHAIN ALKYL CARBOXYLIC ACIDS AND SALTS AND DERIVATIVES THEREOF

## 5 RELATED APPLICATIONS

This application is a continuation-in-part (CIP) of U.S. Serial No. 403,528, filed July 30, 1982, which is a CIP of U.S. Serial No. 354,301, filed March 3, 1982.

#### TECHNICAL FIELD

The present invention relates to chemical compositions which, when applied to growing plant life or seeds thereof, are effective in the growth of said plant life. More particularly, the invention relates to chemical compositions containing long-chain carboxylic acids and salts and derivatives thereof in combination with metal ions which are useful in stimulating plant growth.

#### BACKGROUND ART

In recent years, long-chain carboxylic acids and their derivatives have become recognized as plant growth regulating agents, however, the practical utility of these compounds has been restricted as herbicidal agents, or chemical agents which otherwise inhibit plant growth. U.S. Patent No. 3,180,750 to Davidson, et al., describes partially esterified carboxylic acids having at least two carbon atoms which exhibit herbicidal activity to kill or



severely damage plant life. U.S. Patent No. 3,619,165 to Covey, et al., outlines the use of alkynyl carboxylates for killing meristemic buds, said alkynyl carboxylates having alkyl groups containing five to sixteen 5 carbon atoms. U.S. Patent 3,619,168 to Mecklenborg describes the herbicidal utility of long-chain acids and esters with chain lengths between four and twenty-two carbons. U.S. Patent No, 3,620,712 to Conklin also describes carboxylic acids with chain lengths between 10 six and twelve carbon atoms as herbicidal agents. Others have described other long-chain acids and esters for similar herbicidal use, such as Darlington in U.S. Patent No. 2,117,856 and Stewart, et al., in U.S. Patent No. 2,603,560, the former describing compounds having carbon 15 chain lengths of twelve or less, and the latter, chain lengths of one to fourteen carbons. No reference to longchain carboxylic acids or salts or derivatives thereof which show practical utility as plant growth regulating agents was found in the literature by the present inventor 20 which have a chain length of more than twenty-two carbon atoms. Also, no references are found relating to plant growth-stimulating effects of said compounds.

Long-chain alcohols have been recognized as useful plant growth-regulating compounds, such as n-dodecanol for 25 the removal of suchers from tobacco. Only one long-chain alcohol, however, has been found to stimulate the growth of plant life, viz., 1-triacontanol, a thirty-carbon, straight-chain alcohol, as described by Ries, et al., in U.S. Patent No. 4,150,970, and Welebir, in U.S. Patent No. 30 4,333,758. The initial inconsistency of results described by Ries and co-workers led the present inventor to investigate alternative formulations of 1-triacontanol, and U.S. Patent 4,333,758 teaches that the use of metal ions in the formulations shows a synergisite effect on the 35 activity of the alcohol, and increases its consistency in promoting plant growth.

Long-chain carboxylic acids, such as 1-triacontanoic acid, have been investigated, together with derivatives

thereof, as possible plant growth regulators, however, all results heretofore have proved negative. For example, Jones, et al., have reported that both 1-triacontanoic acid and methyl octacosanoate are both ineffective as plant growth regulators (Planta, 144: 277 (1979)), and 1-triacontanoic acid and other similar compounds were found ineffective in altering the growth of wheat (Charlton, et al., Can. J. Plant Sci., 60: 795 (1980)).

# DISCLOSURE OF THE INVENTION

The present inventor has discovered, most 10 surprisingly, that combining long-chain carboxylic acids having about 12 or more carbon atoms with metal ions having a valence of +2 or more, or salts, esters, or other derivatives of said carboxylic acids with said metal ions, produces compositions which are remarkably effective 15 in the stimulation of plant growth, and indeed superior to those observed with 1-triacontanol formulations containing metal ions, as previously described in his U.S. Patent 4,333,758. Also, in most cases, carboxylic acids, or salts, esters, or other derivatives thereof, 20 having somehwat less than thirty carbon atoms are found to have a superior effect over 1-triacontanoic acid. Furthermore, the compounds of the invention may be combined with other plant growth substances in order to alter their effects on plant life, or may be used 25 simultaneously with herbicidal agents or fertilizers in order to lower the cost of applying each individually.

Previous teachings by the present inventor have disclosed that 1-triacontanol formulations are effective at alkyaline pH, and are affected by fertilizers and temperature. Using the compositions of the present invention, however, these are of little concern, and improved results are obtained at different optimum temperatures than those which are optimum when 1-triacontanol is applied, and responses in the presence of certain fertilizers is also found to be different than those found using triacontanol formulations. Due



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to these facts and others which will become clear in the following detailed description of the invention, the mechanism by which the compositions of the present invention may exert their effects appear to be quite different to that whereby triacontaol exerts its effects on plant life. Furthermore, natural products containing the long-chain compounds of the invention may be used. While some of these also are found to contain a relatively small quantity of triacontanol, a compound ubiquitous in nature, the effects of the naturally-occurring compounds in the compositions of the present invention are shown to be due to the compounds of the invention and not to triacontanol.

Accordingly, it is an object of the present invention to provide an inexpensive and effective means of stimulating the growth of plants and increasing the yield of crops in the field.

It is a second object of the invention to provide novel formulations of carboxylic acids, and their salts and derivatives, and metal ions which are highly effective in stimulating plant growth and enhancing crop yields.

It is a third object of the invention to provide methods of application of the compounds of the invention to the area where plants are growing.

It is a fourth object of the invention to provide an environmentally safe means of stimulating plant growth and crop yield.

It is a fifth object of the invention to provide a convenient method of stimulating plant growth and crop yields.

Pursuant to the present invention, the above problems have been substantially overcome by providing simple, inexpensive, and safe methods and compositions of long-chain carboxylic acids, esters and salts and derivatives thereof with metal ions, which are useful for stimulating the growth of plant life and increasing the yield of crops in the field.



The composition of the present invention includes at least one compound of the formula:

R-COOR' (I)

or a salt thereof or a derivative thereof wherein R is

5 a straight or branched, substituted or unsubstituted alkyl
group having at least 12 carbon atoms, preferably between
15 to 47 carbon atoms and more preferably between 17 to
35 carbon atoms, R' is hydrogen or a straight or branched,
substituted or unsubstituted alkyl group having between 1

10 to 36 carbon atoms, preferably 1 to 30 carbon atoms, and
more preferably 1 to 24 carbon atoms; and a metal salt
wherein the metal ion of said salt in an aqueous solution
has a valence of +2 or +3, said metal salt being present
in said formulation in an amount effective to assist

15 said compound in stimulating plant growth.

While straight chain saturated carboxylic acids and esters thereof are preferred, unsaturated acids and esters are also yery useful, with practical utility diminishing with the number of carbon-carbon double bonds in the straight chain. If R or R' are unsaturated, it is 20 desirable that there be 1 to 6 double bonds, preferably 1 to 3 double bonds and most preferably only 1 double bond. It is preferred that R and R' both be straight chain alkyl groups, however, compounds wherein R and/or 25 R' are branched are also useful in accordance with the present invention. If the Compound (I) is a carboxylic acid, monobasic carboxylic acids are preferred. However, dibasic and polybasic acids show comparable activity to monobasic acids and the number of carboxyl groups attached to hydrocarbon chain influences activity to a 30 lesser degree than the number of carbon-carbon double bonds in the straight chain of the carboxylic acid.

The compounds of the present invention are preferably of the formula:

R-COOR' (I)

or a salt thereof wherein R is a saturated long-chain alkyl group having 15 to 47 carbon atoms and R' is hydrogen or a saturated alkyl group having between 1



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to 36 carbon atoms.

While R' may be over 36 carbons in length, these compounds are considerably more expensive to produce than the shorter chain analogs. Thus, 1-tetracosanyl tetra-5 cosanoate shows superior effects than tetracosanoic acid, and is also superior to 1-methy1 tetracosanoate. not intending to be bound to the mechanism whereby the invention achieves its remarkable results, the longer the chain contributed by the R' group, the better the hydrophobic bonding to a plant "receptor" may be. Other derivatives of carboxylic acids are useful in carrying out the present invention, such as anhydrides, carbohydrate esters and the like, thiocarboxylic acids and esters and salts thereof, cholesteryl esters or other steroidal esters, amides, triglycerides, and other related compounds. Any compounds which may release the free carboxylic acid, or salts or derivatives thereof, in aqueous solution by the action of acids or bases or other means are also within the scope of the present inventoin. Salts of the carboxylic acids of the invention are also highly useful, and show a higher solubility in aqueous solution than the free acids or esters.

Specific examples of saturated acids of the formula (I) include  $CH_3(CH_2)_{16}COOH$  (Stearic acid),  $CH_3(CH_2)_{18}COOH$ (Eicosanoic acid), CH3(CH2)20COOH (Docosanoic acid),  $CH_3(CH_2)_{22}COOH$  (Tetracosanoic acid),  $CH_3(CH_2)_{24}COOH$ (Hexacosanoic acid), CH3(CH2)26COOH (Octacosanoic acid), and  $CH_3(CH_2)_{28}COOH$  (Triacontanoic acid). Of the above compounds, eicosanoic acid, tetracosanoic acid and triacontanoic acid are preferred.

Specific examples of saturated esters of the formula (I) include  $CH_3(CH_2)_{28}COOCH_3$  (Methyl triacontanoate),  $CH_3(CH_2)_{34}COOCH_3$  (Methyl hexatriacontanoate),  $CH_3(CH_2)_{40}COOCH_3$  (Methyl dotetracontanoate),  $CH_3(CH_2)_{46}$  $COOCH_3$  (Methyl octatetracontanoate),  $CH_3(CH_2)_{22}COO(CH_2)_{23}$  $\mathrm{CH}_3$  (Tetracosanyl tetracosanoate), and  $\mathrm{CH}_3(\mathrm{CH}_2)_{22}$ - $COO(CH_2)_{29}CH_3$  (Triacontanyl tetracosanoate). Of the above compounds, Methyl triacontanoate, Tetracosanyl

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tetracosanoate and Triacontanyl tetracosanoate are preferred.

Examples of unsaturated esters of the formula (I) include  ${\rm CH_3(CH_2)_{10}CH=CH(CH_2)_4COOCH_3}$  and  ${\rm CH_3(CH_2)_{22}^-}$  COO(CH<sub>2</sub>)<sub>4</sub>CH=CH<sub>2</sub>.

Examples of unsaturated carboxylic acids of the formula (I) include  ${\rm CH_3(CH_2)_7CH=CH(CH_2)_7COOH}$  (oleic acid) and  ${\rm CH_3(CH_2)_{12}CH=(CH_2)_9COOH}$  (nervonic acid).

Examples of polybasic carboxylic acids include HOOC(CH<sub>2</sub>)<sub>16</sub>COOH (1,18-octadecanedioic acid), HOOC(CH<sub>2</sub>)<sub>20</sub>-COOH (1,22-docosanedioic acid), HOOC(CH<sub>2</sub>)<sub>22</sub>COOH (1,24-tetracosanedioic acid) and HOOC(CH<sub>2</sub>)<sub>10</sub>CH(CH<sub>2</sub>)<sub>10</sub>COOH (1,12,24-tetracosanetrioic acid).

Examples of salts of the compounds of the formula (I) include, but are not limited to, salts having the formula

wherein X is  $Ca^{+2}$ ,  $Na^{+2}$ ,  $K^+$ ,  $Mg^{+2}$ ,  $La^{+3}$ ,  $Mn^{+2}$ ,  $Zn^{+2}$ ,  $NH_4^+$ , anilinium, octadecyl ammonium, and the like wherein n is the valence of the cation.

According to the present invention, the compounds of the invention are solubilized in an aqueous solution containing at least one metal ion having a valence of +2 or more. The compounds may be dispersed in aqueous solution by any method practiced in the art, including simple solubilization of the compound in the aqueous solution by stirring, heating, and the like, or may be first dissolved in an organic solvent which is subsequently dissolved in a relatively large amount of water, with or without the aid of a surfactant, and preferably in a surfactant-free medium. The most preferred method of solubilizing the compounds of the present invention in aqueous solution comprises coating the carboxylic acids, or salts or derivatives thereof, or combinations thereof, on the dry salts of the metal ions by means of a solvent in which any of the compounds being used to coat the metal ion salts are soluble.



Subsequent dilution in water affords a highly useful solution of the compounds of the invention with metal ions.

When the composition of the present invention is in a dry form, the weight ratio of said compound to said metal salt is in the range of 1:1 to 1:5,000,000,000, preferably 1:5 to 1:500,000,000, more preferably 1:100 to 1:50,000,000 and most preferably 1:1,000 to 1,20,000,000. Optimum weight ratios will vary some—

10 what depending upon the particular compounds of the formula (I) and metal salts which are utilized. The optimum weight ratio will also vary somewhat depending upon the type of plants which are being treated therewith.

The composition of the present invention contains 15 the compound of the formula (I) and the metal salt in an amount effective to stimulate plant growth. the composition is in dry form, the composition will usually contain the metal salt in an amount of 50 grams to 1000 grams per kilogram, preferably 250 grams to 20 1000 grams per kilogram, most preferably 500 grams to 1000 grams per kilogram of the composition. The compound of the formula (I) will be preferably contained in an amount of 0.1µg to 10g per kilogram, preferably 50µg to 5g per kilogram, most preferably lug to lg per kilogram 25 of the composition. As discussed hereinbelow, the composition may contain additional active ingredients which improve or do not substantially inhibit the plant growth stimulating effects of the composition of the present invention. The composition may also contain 30 various inert ingredients (liquid or solid) which are incorporated into the composition in varying amounts depending upon the mode of application to plant life.

Typically, one part by weight of a compound of the invention, or mixtures of more than one compound, is diluted to a final solution with up to about 5,000,000,000 parts of water (by weight), preferably diluted with between about



1 4,000 to 2,000,000,000 parts of water, and more preferably with between about 40,000 and 200,000,000 parts of water.

Thus, the effective range of concentrations of the compounds of the invention in solution with metal ions may vary widely while achieving similar results in stimulating plant growth and the yield of crops in the field.

If an organic solvent is used to form a concentrate of the carboxylic acids or derivatives thereof, the compound is first dissolved in the organic solvent, with or without the application of heat. The resulting solution is then 10 added to water, which may contain the metal ions of the invention, or the metal ions may be added after the addition of the concentrate. Typically, one part (by weight) of one or more of the carboxylic acids or derivatives thereof of the invention is dissolved in between about one to 5,000,000 parts 15 of polar organic solvent (if no surfactant is to be added, or relatively nonpolar solvent if a surfactant is to be added), preferably one or more of the said compounds of the invention is dissolved in between about 10,000 and 500,000 parts of polar solvent (by weight), and more preferably, 10,000 parts to about 160,000 parts of solvent may be used, and most preferably between about 10,000 and 80,000 parts of solvent may be used. If a nonpolar solvent is employed, then one part of one or more compounds of the invention (carboxylic acids, salts, esters, or other derivatives thereof), by weight, 25 may be dissolved in between about one and 10,000 parts of nonpolar solvent, preferably between about 10 parts and 1,000 parts of nonpolar solvent, and more preferably between about 100 and 1,000 parts of nonpolar solvent (by weight). resulting solution is then dissolved in water as previously 30 described, with or without the use of a surfactant additive, depending on the solubility of the solvent which would be required in water to attain the desired concentration of the compounds of the invention.

Polar organic solvents which are useful in carrying 1 out the present invention in order to aid in the solubility of the long-chain compounds of the invention include, but are not limited to, ketones, alcohols, water-soluble ethers, glycols, sulfoxides, organic carboxylic acids of relatively low molecular weight, amines, dipolar, aprotic solvents such as DMSO (dimethyl sulfoxide), DMF (dimethyl formamide), and HMPA (hexamethyl phosphoramide), and the like. Typical polar organic solvents include acetone, methyl ethyl ketone, diethyl ketone, cyclohexanone, methanol, ethanol, propanol, isopro-10 panol, butanol, isobutanol, t-butanol, sec-butanol, ethylene glycol, propylene glycol, diethylene glycol, glyme, diglyme, dioxane, tetrahydrofuran, acetic acid, formic acid, propionic acid, lower aliphatic amines, and other similar solvents which show a solubility in water and in which the compounds 15 of the invention are also soluble at concentrations where the resulting concentrate is useful in stimulating plant growth and crop yields when diluted to a final volume in water.

Nonpolar organic solvents which may be employed to aid in the dispersion of the compounds of the invention with or without the aid of a surfactant include, but are not limited to, hydrocarbons, higher alcohols, aromatic hydrocarbons, water-insoluble ethers, esters, amines, halogenated hydrocarbons, and the like. Typical nonpolar solvents 25 include chloroform, methylene chloride, carbon tetrachloride, freons, benzene, toluene, xylenes, aniline, pentanols, hexanols, heptanols, octanols, other long-chain alcohols, pentane, hexane, heptane, other hydrocarbon solvents, both aliphatic and aromatic, alkenes, alkynes, higher aliphatic amines, ethyl acetate, amyl acetate, other lower esters, and 30 other nonpolar compounds in which the compounds of the invention are soluble, and which further may be dispersed in water containing metal ions, or before said metal ions are added to the solution, either with or without the aid 35 of surfactant additives.

Surfactants useful in dispersion of the compounds of the invention in aqueous media include, but are not limited to, Tweens, long-chain alkyl sulfonates, Zonyl surfactants, alkyl sulfates, nonionic surfactants, anionic surfactants, cationic surfactants, and other surfactants known in the art which are useful for dispersion of essentially nonpolar solvents or compounds in aqueous media. If surfactants are employed, they may be added either prior to or after the addition of metal ions to the aqueous solution, or prior to or after the addition of the solution of the compounds of the invention in an organic solvent to the aqueous portion of the formulation. The amount of surfactant used is preferably kept to a minimum, since complexation or precipitation of the metal ions may occur, thereby lowering the growth promoting effect, or causing additional metal ions to be added. Typically, surfactants are used up to a concentration (volume/volume) of about 5%, with a concentration of between about 0.1 and 3% being preferred, and a concentration of between about 0.1 and 1% being most preferred.

Metal ions of the invention, which, in combination with the long-chain carboxylic acids, and salts and derivatives thereof, produce the remarkable growth-stimulating effects, include any cation having a valence of +2 or more. While metal ions of lower valence produce a small effect, a higher valence is required for the full growth-stimulating effect. Typical metal ions useful in carrying out the present invention include, but are not limited to, Ca<sup>+2</sup>, Ba<sup>+2</sup>, La<sup>+3</sup>, Cd<sup>+2</sup>, Pb<sup>+2</sup>, Co<sup>+2</sup>, Mn<sup>+2</sup>, Ce<sup>+4</sup>, Mg<sup>+2</sup>, Zn<sup>+2</sup>, Cu<sup>+2</sup>, Fe<sup>+3</sup>, Fe<sup>+2</sup>, Ni<sup>+2</sup>, and the like, however, only a limited number of metal ions are preferred due to their superior effect and relatively low toxicity. Metal ions of higher toxicity, such as Pb<sup>+2</sup> and Cd<sup>+2</sup> are very useful, and may be used on plant life not utilized for food purposes. Other metal ions, such as Sr<sup>+2</sup>, are especially useful, however, the most preferred metal ions



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1 are Ca<sup>+2</sup>, La<sup>+3</sup>, Mg<sup>+2</sup>, and Mn<sup>+2</sup> due to their low toxicities. Furthermore, Ca<sup>+2</sup> is inexpensive, and useful salts are exempt from tolerance requirements by the U.S. Environmental Protection Agency. While some of these metal ions are

known to affect the response of plants to the known plant growth substances, these belong to the well-known Hofmeister series, and include Ca<sup>+2</sup>, La<sup>+3</sup>, Mg<sup>+2</sup>, and Mn<sup>+2</sup>. The activity observed with other polyvalent metal ions, however, indicates that the biological activity of these cations in the compo-

sitions of the present invention may not be related to the known effects of the Hofmeister series cations. While the precise mechanism remains unclear at present, the metal ions of the present invention, together with the compounds of the invention, were found inactive when applied separately -- not in combination -- under identical conditions of application.

In the preferred embodiment of the present invention, the compounds of the invention are either (1) added to a solution of metal ions in water in a polar organic solvent, or (2) coated on the dry salts of the metal ions of the invention by use of a suitable solvent which is subsequently allowed to evaporate, with or without the use of heat. The latter method is most preferred, since it allows for a product requiring only a single container without the need for a separate package containing a solution of the long-chain compounds. Since the long-chain compounds may be used at a very low concentration, solubility usually poses no problem when the dry product is added to water.

Preferred metal ion concentrations in the final/composition which is applied to plant life are between 0.1 and 50 mM, with concentrations of between about 1 and 30 mM being more preferred. If a surfactant is part of the final formulation, higher metal ion concentrations may be required, and may extend up to about 1 molar.



In accordance with another aspect of the invention, other plant growth substances appear to alter the effects of the compounds of the invention, such as auxins, gibberellins, cytokinins, abscisic acid, ethylene, together with salts and 5 synthetic analogs thereof. Auxins, in particular, are capable of extending the useful range of metal ion concentrations which stimulate plant growth in the compositions of the present invention. This is of practical utility on crops which respond at relatively low metal ion concentrations, such as field corn, where hard water used for spraying may 10 contain high levels of metal ions. Auxins include all natural and synthetic auxins, with auxins such as indole-3acetic acid (IAA) and naphthalene acetic acid (NAA) being among the preferred auxins. These may be added to the solutions of the compounds of the invention, or to the 15 aqueous solution which may contain the metal ions of the invention, with or without the addition of the compounds of the invention. Alternately, they may be coated on the salts of the metal ions by any method practiced in the art. 20 Water-soluble salts of the auxins, gibberellins (such as gibberellic acid and the like), and cytokinins (such as kinetin, benzyladenine, and the like) are especially useful.

When applied to the area where plants are growing, the long-chain carboxylic acids, or esters, salts, or derivatives thereof, alone or in combination, it is desirable to apply 25 at least 0.01 mg of the compounds per acre of land, with at least 0.03 mg of the compounds applied per acre being preferred. Up to several grams of the compounds may be applied per acre of land, however, this is not usually 30 necessary, and a limited advantage through increasing the application rate is observed, as will become apparent in following examples of the invention. The preferred mode of applications of the compounds of the invention in solution with polyvalent metal ions is foliar spraying of the formulations as a fine mist onto the leaves of plant life.



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l activity has been observed using other means of application, such as seed soaks, and soil drenches. Seed soaks have the advantage of requiring a small volume of the compositions of the invention, while soil drenches require considerably larger quantities. For seed soaks, the preferred ratio of seed to solution of the compounds of the invention including metal ions in solution is at least 2 parts solution to 1 part of seed (volume/volume), and similar results are achieved at higher solution to seed ratios, extending to about 25 to 1 or more. Soil drenches require a considerable quantity of 10 solution, amounting to between about 1 ml per plant to 10 liters per plant, with 1 ml to 1 liter per plant being preferred, and 5 ml to 1 liter per plant being more preferred. Of course, larger plants require larger volumes of solution 15 than smaller plants, and plants with deeper root systems likewise require more solution to be applied to the soil in which they are growing. Combinations of soil drenches, seed soaks, and foliar application are also useful, and other modes of formulation or application, such as formations 20 of coloidal suspensions of the compounds of the invention, and the like, applied as innoculants, use of the formulations as fertilizer additives or complements, and the like, are not considered to be beyond the scope of the present invention.

The formulations of the present invention are useful on
25 plant life at all stages of development, with some plants
responding better at relatively early stages as compared to
other plants. The preferred stage of development is at the
stage where the plant bears between about two and seven true
leaves (or sets of two leaves each in the case of dicots, or
30 up to the fifth trifoliate stage on beans and soybeans), with
the preferred stage between three and six true leaves. For
grasses, such as wheat, corn, sorghum, and the like, later
stages of growth have shown some advantage. Corn may be
treated at a stage before or after tassel initiation in the
35 plant. Wheat is best treated in the spring rather than soon



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1 after germination in the fall. Perennial plant life may be treated seasonally, with treatments yearly in the spring being preferred. Multiple sprayingson growing plants has also indicated that improved results may be obtained in this manner, especially on soybeans, tomatoes, and the like.

While the pH of the final formulation is of no concern, best results are obtained where the degree of acidity or alkalinity is not severe, preferably between pH 4 and 12, and more preferably between pH 5 and 10. Due to the tolerance of the metal ion concentrations for most plants, hard water (or well water) may usually be used without concern for metal ion concentration in the water. Highly poluted water, or that known to contain large concentrations of hydrocarbons or other long chain compounds (water in which oil has formed on the surface, e.g.) should be avoided.

Plants are best fertilized using normal agricultural practices, with no unusual or additional requirements, and results are observed even at suboptimum levels of fertilizers. In greenhouse trials, however, the use of concentrated water-

- 20 soluble fertilizers affects results somewhat, and some guidelines for greenhouse trials are given in the following examples of the invention. Improved results have also been observed with the inclusion of foliar fertilizers into the compositions of the present invention. These include water-
- 25 soluble salts which supply plants with essential nutrients, micronutrients (including the metal ions of the invention), nitrogen containing compounds, such as urea, ammonium nitrate, and the like, potassium, and other compounds or combinations thereof known in the art. Compounds which complex or
- oprecipitate the metal ions of the invention produce lesser increases when combined in solution with the compounds and metal ions of the invention, and therefore show somewhat more limited practical utility.

The metal ions of the invention are any metal ions having 35 a valence of +2 or more, and those released from inorganic



metal salts are preferred, however, any organic or inorganic salt or compound capable of releasing an effective concentration of the desired metal ions in aqueous solution may be used. Counter ions, such as Cl, NO<sub>3</sub>, acetate, and the like, are of minimal importance, since these do not appear to influence the activity of the cations of the invention. Also, some complexed metal ions may be useful, as well as aquated metal ions.

Salts of the carboxylic acids of the present invention are very useful in carrying out the methods of plant growth 10 stimulation of the invention, and show improved solubility over the free acids of the invention. These salts may be any known in the art which show a solubility in the concentration range useful for the carboxylic acids of the The salts of alkali metals show preferred 15 invention. water solubility, however, since the compounds of the invention show solubility at the low concentrations which are useful, described further henceforth, other metal salts are also of valuable use, such as salts of the metal ions of the invention. These polyvalent ions show 20 a limited solubility at certain concentrations, when combined with long-chain carboxylic acids, however, it is important to note that the concentrations of the carboxylic acids of the invention which show effective results may be low enough to present no solubility in aqueous solutions 25 prepared therefrom. Also, salts of carboxylic acids, or derivatives thereof, may include a plurality of metal ions, and metal ions useful in carrying out the invention may be in the forms of salts of a plurality of long-chain compounds of the invention. 30

It has further been found that different species of plant life respond optimally at somewhat different concentration of metal ions while concentration of the long-chain compounds of the invention remains at a much broader range. For example, soybeans, sweet corn, and a large variety of vegetables respond to metal ion concentrations



- 1 over a broad range of between about 5 mM to 15 mM or more. In some cases, positive results are seen at lower metal ion concentrations. Field corn appears to respond well at relatively low concentration of metal ions, i.e. between
- 5 about 1 mM and 4 mM, with between about 2 mM and 3 mM being preferred. Depending on the cultivar used, this range may be somewhat broader. The invention is further distinguished from the results observed in the prior art (e.g., U.S. Patent 4,333,758 to the present inventor) since
- the use of formulations containing metal ions and 1triacontanol is severely limited for use on field corn,
  with responses being observed only on a few cultivars at
  a low, narrow range of metal ion concentrations, viz.,
  between about 1.00 and 1.25 mM. The compositions of the
- present invention show positive results on all cultivars of field corn tested with a similar formulation containing metal ions in the concentrations described above.

Other crops, such as peas and the like, respond to formulations of the compounds of the invention containing higher metal ion concentrations. These crops are seen to respond well at metal ion concentrations ranging up to 20 mM and more, while wheat responds well at concentrations over about 15 mM to 20 mM. From the detailed description given hereinafter, optimum concentrations of metal ions, together with concentrations of the compounds of the invention, which enhance the growth of many crops and other plants will become apparent.

The formulations of the present invention are useful on a very large variety of plants, and may be applied in a variety of ways. Crops which respond favorably are included in the group comprising, although not limited to, field corn, popcorn, sweet corn, milo, sorghum, wheat, barley, oats, rice, rye, apples, crabapples, pears, quinces, avocados, papayas, blackberries, dewberries, loganberries, raspberries, blueberries, currants, gooseberries, huckleberries, cherries, plums, prunes, oranges, citrus citron, grapefruit, kumquats, lemons, limes, tangelos, tangerines, mangoes, persimmons, peaches, apricots, nectarines, beans, peas, soybeans, broccoli, brussels sprouts,

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1 cauliflower, kohlrabi, cantaloups, honeydew melons, muskmelons, pumpkins, watermelons, winter squash, carrots, garden beets, sugar beets, horseradish, parsnips, radishes, rutabagas, salsify roots, turnips, celery, fennel, 5 cucumbers, summer squash, lettuce, endive (escarole), chinese cabbage, salsify tops, onions, garlic, leeks, shallots, potatoes, Jerusalem-artichokes, sweet potatoes, yams, spinach, beet tops, collards, dandelion, kale, mustard greens, parsley, Swiss chard, Turnip tops, water-10 cress, sunflowers, tomatoes, eggplants, peppers, pimientos, pecans, almonds, brazil nuts, bush nuts, butternuts, chestnuts, filberts, hazelnuts, hickory nuts, walnuts, alfalfa, Bermuda grass, bluegrass, clovers, cowpeas and cowapea hay, fescue, lespedeza, lupines, orchard grass, 15 peanut hay and peanuts, cashews, peavine hay, rye grass, soybean hay, sudan grass, timothy, vetch, corn forage, sorghum forage, sugar cane, cane sorghum, bananas, pineapples, grasses and grain used to feed livestock, crops used to feed poultry, fish, and other animal life, pawpaws, 20 and other raw agricultural commodities.

The formulations of the present invention may also be useful in enhancing the growth and quality of trees, such as loblolly pines, Australian pines, Douglas fir, pine trees, oak trees, and other trees. The formulations of the invention are similarly useful for use on ornamental plants, including any plant used indoors or outdoors for ornamental purposes, and may be expected to show some effect on the growth of fungi under appropriate conditions. Also, compounds of the invention which show mammalian activity or other activity in life forms may be expected to show improved activity when combined with the metal ions of the invention.

In accordance with an additional aspect of the present invention, naturally-occurring products which contain active amounts of the compounds of the invention are highly useful when combined in the formulations with metal ions. In particular, these include naturally-occurring waxes and oils and the





- l like, including compounds of the invention, or mixtures thereof, which are obtainable from natural sources. These usually include useful compounds such as the long-chain carboxylic acids of the invention, esters thereof, hydroxy acids, and the like, and include both saturated and unsaturated compounds. The compounds may further be present as esters of other naturally-occurring compounds containing carboxyl groups or hydroxyl groups, such as auxins, gibberellins, sugars, etc.
- 10 Useful naturally-occurring compounds include, but are not limited to, candelilla wax, carnauba.wax, chinese insect wax, esparto wax, ghedda wax, Japan wax, peanut oil, olive oil, rice bran oil, shellac, sisal wax, soybean oil, beeswax, etc. The preferred members of the foregoing comprise any naturally-occurring wax, oil, and the like, which contain compounds of the invention, or mixtures thereof, such as beeswax, esparto wax, and the like.

In a preferred embodiment of the present invention, such naturally-occurring waxes are used in the formulations of the invention, and may be used in any manner in which the pure compounds of the invention are utilized. Especially useful are concentrated forms of the formulations, made by coating a relatively small quantity of the naturally-occurring wax, oil, or the like, or mixtures thereof, directly on a metal salt of the metal ions of the invention, by means of an appropriate solvent or other means, as described in the foregoing description.

While these naturally-occurring compounds may contain an additional active ingredient, such as 1-triacontanol,

30 examples in the following best mode of the invention are given illustrating that this compound is <u>inactive</u> in the formulations as they are described herein. Many compounds and other substances are used in agricultural practice which also contain 1-triacontanol, however, the 1-triacontanol is recognized as essentially inactive. These include mulches, cow manure, paper, peat and peat moss, compost, and the like.

SUBSTITUTE SHEET



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Substances such as these have been in agricultural use since the genesis of agricultural practice, and are impatentable as methods of increasing crop production and plant growth, as opposed to specific formulations of the ingredients contained therein, highly useful in promoting plant growth,

such as are described in U.S. Patent No. 4,150,970 to Ries, et al., and U.S. Patent No. 4,333,758, to the present inventor. Formulations of more purified forms of the compounds of the invention, such as those contained in naturally-occurring plant products, and the like, in combination with specific

plant products, and the like, in combination with specific concentration ranges of added metal ions in aqueous solution, however, offer superior improvements and refinements over any prior art found by the present inventor. The surprising results obtained through use of the formulations of the present invention will become apparent to the skilled artisan in the

following description of the invention.

In accordance with another aspect of the invention, seed obtained from crops sprayed with the formulations of the invention show improved quality and may be expected to show increases in crop yields obtained through the planting of said seed with or without further application of the formulations, and, indeed, additive increases may be observed from growing season to growing season. Improved germination has been observed on seeds of crops which require longer germination time or grow slowly on germination, such as tomatoes, peppers, celery, lettuce, tobacco, and the like.

The invention being thus described, other objects and further scope of the present invention will become apparent from the detailed description given hereinafter. It should be understood, however, that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

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# 1 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph showing a typical dose response curve for the compounds of the invention, illustrated for the response of field corn (cv. Pioneer 3780) to formulations of the invention containing 3 mM CaCl<sub>2</sub> and varying concentrations of 1-triacontanoic acid.

Fig. 2 is a graph showing the optimum hydrocarbon chain lengths of compounds of the invention for use on tomatoes  $(CaCl_2 = 10 \text{ mM})$  and field corn  $(CaCl_2 = 2 \text{ mM})$ .

Fig. 3 is a graph showing the response of field corn seedlings (cv. Pioneer 3535) to formulations of the invention containing beeswax (10  $\mu$ g/liter) and Ca<sup>+2</sup> (2 mM) at different stages of development.

# BEST MODE

#### 15 EXAMPLES

The following examples are presented herein as being exemplary of the present invention and, accordingly, should not be considered, in any way, as being limitative of the applicant's inventive contribution.

In the preferred mode of the invention, compounds of the structure:

#### R - COOR'

wherein R is a long-chain alkyl group containing from 15 to 47 carbon atoms, and may contain between about 0 to 6 carbon-carbon double bonds, and be substituted by about 0 to 4 carboxyl groups or derivatives thereof; and

R' is a long-chain alkyl group having between about 1 and 36 carbon atoms, and may contain between about 0 to 6 carbon-carbon double bonds, and be substituted with about 0 to 4 carboxyl groups, or derivatives thereof, or is hydrogen; are considered the most preferred members of the classes of compounds disclosed in the foregoing Specification. These are used to stimulate plant growth in the formulations given by examples hereinbelow in combination with metal ions having a valence of +2 to +3. The compounds of the invention may also be contained in naturally-occurring oils, waxes, and the like,

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as described in the following description of the preferred embodiments of the invention. Also, compounds hydrolyzed in situ to compounds of the invention in the compositions are not considered beyond the scope of the present invention.

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## FORMULATION 1

A 5 mg quantity of 1-triacontanoic acid was dissolved with heating in 50 mL of acetone. The concentrate was subsequently added to a solution of metal ions in water, and was applied as a foliar spray to plant life.

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## FORMULATION 2

A 5 mg quantity of 1-tetracosanoic acid, or other longchain acid, was added to 50 mL of acetone, optionally heated to dissolve the compound. The solution was added to a solution of metal ions and applied as described for Formulation 1.

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## FORMULATION 3

A 5 mg quantity of a long-chain ester was dissolved in 50 mL of a ketone or alcohol solvent, with or without the application of heat. The solution was added to an aqueous solution of metal ions and applied to plant life, or used a seed soak or soil drench.

FORMULATION 4

A 5 mg quantity of a long-chain, unsaturated carboxylic acid, having at least one carbon-carbon double bond, was dissolved in acetone or another suitable solvent. The solution was subsequently added to a larger volume of water containing metal ions and was applied to plant life.

#### FORMULATION 5

A 150 mg quantity of a long-chain or fatty ester was dissolved in 100 mL of trichloroethylene, with or without the application of heat. An amount of the resulting solution was then added to a solution of metal ions, with or without the aid of a surfactant additive, and sprayed on the leaves of growing plant life.



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# 23 FORMULATION 6

A 50 mg quantity of 1-tetracosanoic acid (or other long-chain acid or ester) was dissolved in 50 mL of acetone or other solvent in which the compound was soluble, with or without the application of heat. The concentrate was subsequently used to coat the salt of a metal ion of the invention, allowed to dry, and the salt then solubilized in water and applied to plant life, or used as a seed soak or soil drench. Weight ratios (compound:salt) are < 1:100.

FORMULATION 7

A 150 mg quantity of a naturally-occurring wax was solubilized in 100 mL of trichloroethylene, with or without the addition of heat. The solution was then utilized to coat salts of metal ions of the invention, which were then dissolved in water to form a formulation with the desired concentration of both components. The solution was then applied as described under Formulation 6, hereinabove.

#### FORMULATION 8

A 150 mg quantity of a naturally-occurring wax or oil solubilized in 100 mL of a nonpolar solvent, such as trichloroethylene, chloroform, benzene, and the like, and the resultant solution was further dissolved to allow sufficient volume resulting therefrom to coat a quantity of a metal ion salt, or combination thereof, relatively larger than that described under Formulation 7 hereinabove. The solvent used to further dilute the concentrated solution may be selected from groups of solvents which are either polar or nonpolar in nature. The resulting treated salts of the metal ions of the invention are then diluted in water after evaporation of the solvent.

#### DETAILED EXAMPLES

# EXAMPLE I

A 0.1 mL quantity of Formulation 1 was added to a solution of MgCl<sub>2</sub>, or other metal salt, at a metal ion concentration of 3 mM in water, stirred, and sprayed on the leaves of field corn seedlings.

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# EXAMPLE II

A 1.0 mL quantity of Formulation 1 was added to a solution of  $CaCl_2$ , or other  $Ca^{+2}$  salt, in water at a concentration of 10 mM (200 mL total volume). The solution was shaken and sprayed on the leaves of soybean seedlings (1-triacontanoic acid concentration 0.1 mg/L).

# EXAMPLE III

A 0.01 mL quantity of Formulation 2, containing stearic acid, was added to a solution of  $\text{Ca}^{+2}$  or  $\text{La}^{+3}$  salts having a metal ion concentration of 10 mM. After stirring, the solution was applied to the leaves of tomato seedlings.

#### EXAMPLE IV

A 1.0 mL aliquot of 1-tetracosanyl tetracosanoate in acetone solution was dissolved in 2 L of water containing 15 mM of metal salts. The resulting solution was sprayed onto the leaves of pea seedlings.

#### EXAMPLE V

A 0.1 mL quantity of erucic acid was dissolved in a suitable solvent, and this was added to 200 mL of water containing metal ions at a concentration of between about 10 mM and 15 mM. The resultant solution was then applied to the leaves of tomato seedlings, or may be used as a seed soak for tomato seeds.

#### EXAMPLE VI

A 40 mL aliquot of hexacosanoic acid, or salt or suitable derivative thereof, in solution according to Formulation 2 or 2, was added to 10 U.S. gallons of water containing metal ions at a concentration of 3 mM. The final solution was applied as a mist to 1 acre of field corn.

#### EXAMPLE VII

A 0.5 mg quantity of 1-tetracosanoic acid was added to 109 g of anhydrous CaCl<sub>2</sub> and the mixture was allowed to dry. The granular product was dissolved in 100 U.S. gallons of water and applied to 10 acres of field corn as a fine foliar spray, said water having a metal ion concentration of between about 2 and 3 mM.

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EXAMPLE VIII

Beeswax or esparto wax was dissolved in a nonpolar solvent, such as trichloroethylene or the like, according to Formulation 7. The solution was subsequently coated evenly on about 80 kg of anhydrous CaCl<sub>2</sub> and allowed to dry. Dilution of the final product in water to 2 mM Ca<sup>+2</sup> concentration gave sufficient solution to cover 950 acres of field corn.

## EXAMPLE IX

EXAMPLE X

A 0.33 mL aliquot of the solution described in Formulation 7 was added to 109 g of technical grade (77%) CaCl<sub>2</sub>, and the mixture was dried. After dissolving the granular product in 10 U.S. gallons of water, the solution was sprayed at the rate of 10 gallons/acre on one-half acre in two applications.

A 0.67 mL aliquot of the solution according to Formulation 8 was added to 50 mL of acetone (or other solvent), and the solvent was added to 1,090 g of technical grade CaCl<sub>2</sub>. After the product had dried, it was dissolved in 200 gallons of water and applied to 10 acres of a vegetable crop at the rate of 20 gallons/acre.

While the above Examples briefly illustrate the preferred embodiments of the invention only partially, they are not intended to limit, in any way, the scope of possible combinations of formulations and methods of the present invention. The formulations of the invention are applied, preferably to the leaves of growing plant life, and are also useful as seed soaks, soil drenches, and the like. The methods used to evaluate the efficacy of the foregoing Examples of the invention are outlined in the following description of the methods of the invention.



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#### 26 METHODS

The compounds of the invention were obtained from the following sources: straight-chain, saturated and unsaturated carboxylic acids up to thirty carbon atoms were purchased from Sigma Chemical Co., St. Louis, Mo. Long-chain esters were synthesized either by esterification with ethereal diazomethane, or through the reaction of the acid chloride with an alcohol. Amides were prepared through the reaction of acid chlorides with aqueous ammonia. Carboxylic acids and esters with a carbon chain longer than thirty carbons were prepared in accordance with the methods disclosed in either U.S. Patent No. 4,167,641, or those described in the Ph.D. Thesis by the present inventor, entitled "The Synthesis of Long-Chain Carboxylic Acids Containing up to Forty-Eight Carbon Atoms and the Use of Their Potassium Carboxylates as Novel Surface Active Agents, The American University, Washington, D.C., May, 1978.

In order to illustrate the efficacy of the formulations 20 of the invention, two primary methods of forming the formulations were used: (a) solubilizing the compounds of the invention in an appropriate solvent, with polar solvents being preferred over nonpolar solvents, with or without the aid of surfactant additives, and dissolution of the concentrate thus obtained in water, optionally containing a surfactant; and (b) adding 25 the solution of the compounds of the invention described above to the salts of the metal ions of the invention, followed : by evaporation of the solvent. The resulting granular product wasthen dissolved in a desired amount of water to achieve the 30 desired concentration of metal ions in solution. In the cases where the pH of the solution was adjusted, this was accomplished by addition of an alkali, such as sodium hydroxide, to the water prior to dissolution of the concentrated form of the compounds of the invention.



In greenhouse trials, plants were sown in pots 10 to 15 cm in diameter containing Peat-Lite. Twice the number of seeds per plot were sown than the actual number of plants used with the various treatments, with plants being thinned to those of similar size prior to application of the formulations of the invention. In general, seedlings were fertilized on germination with a water-soluble 15-30-15 fertilizer, and again the day following application (15-30-15 fertilizer in the case of dicots, and 30-10-10 fertilizer for monocots).

10 Each plot received ca. 50 mL of fertilizer at a concentration of 2.5 g/L.

In field trials, seeds were sown using standard agricultural practices or as otherwise indicated. Small plots were generally employed, and were carefully selected to minimize variation in plant size within each block. In all trials conducted (greenhouse and field trials), treatments were randomly assigned within each block using standard methods practiced in the art. Plants were blocked by size in the greenhouse trials to minimized the variation due to plant size.

Results obtained were found similar whether plants were grown under natural or artificial lighting (ca. 750 to 1000 ft-can) for many plants tested. When plants were grown under artificial lighting, 26°C day temperatures were maintained with night temperatures of about 17°C. Plants grown by natural lighting were grown at the same time of year as normal for that particular plant in the field. When applications were made at varying temperatures, indoor temperatures were adjusted accordingly, and plants grown outdoors were sprayed at varying times of the day when the desired temperatures were reached.



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Plants were sprayed at various stages of development with formulations of the present invention as described in the examples given hereinafter. In all cases, randomized complete block designs were used, with each treatment being replicated between about 4 to 6 times. In greenhouse trials, plants were generally harvested at least four days after treatment, including roots, and soil was removed therefrom by washing with water. Fresh weights were obtained for each plot, and plants were dried to constant weight in an oven at a temperature below 100° C. Water content values were obtained by subtraction of dry weights from fresh weights, however, dry weights were used as the true indication of growth increases of treated plots over control plots.

In field trials, increases in marketable yield were

determined, and observations were recorded regarding increases in number of fruit, size of grain, and the like. All data were analyzed statistically by standard analysis of variance methods widely practiced in the art, and using Duncan's Multiple Range Test (Biometrics, 11: 1 (1955)). Increases in the dry weights of seedlings found in greenhouse trials were found particularly useful in predicting the yields of crops in field trials, and, in general, dry weights thus obtained may be expected to be lower than the actual yields found in the field.

25 Trials included determination of optimum stages of plant development for spraying or other mode of application of the compounds of the invention, optimum application rates, optimum temperatures, and other conditions which are useful in the application of the compositions of the invention to plant life in the field, greenhouse, or other environment.



invention.

29 Compounds of the invention were tested in solution 1 with the metal ions of the invention, either singularly or in combination with other compounds or metal salts of the The preferred mixture of compounds used was purified beeswax, which is known to contain esters of straightchain monohydric alcohols with even-numbered carbon chains from  $C_{24}$  to  $C_{36}$  esterified with straight-chain acids also having even numbers of carbon atoms up to  ${\rm C}^{}_{36}$  (some  ${\rm C}^{}_{18}$ hydroxy acids). Also contained therein are hydrocarbons with straight carbon chains from  $C_{21}$  to  $C_{33}$  (inert), as described in Merck Index, 9: 1027 (1976). The long-chain esters are also found superior to the carboxylic acids, and the small amount of 1-triacontanol constituent in the wax is also shown in the following detailed description to be inert in the formulations of the present invention containing beeswax. Other naturally-occurring oils, waxes, and the like, are also useful, including those obtained from both plant and animal

The data in the following tables and examples of the invention are intended to be exemplary of the results that can be expected by applications of the compositions of the present invention on plant life using the methods outlined herein, however, the specific compounds, formulations, and methods used in the examples are not intended to be limitative, in any way, of the spirit and scope of the invention as fully described herein.

sources, as described in the foregoing description of the



1 TABLE 1. Increases in the Dry Weight of Field Corn
Seedlings (cv. Pioneer 3780) Treated 7 Days
after Germination with and without 1-Triacontanoic
Acid (0.1 mg/L, Formulation 1) and Naphthalene
Acetic Acid, and with Varying Concentrations of
CaCl<sub>2</sub> (pH = 9, Spray Temperature = 25° C).
Seedlings were Harvested 4 Days after Spraying.

	TRIACON- TANOIC ACID	CaCl <sub>2</sub> (mM)	NAA (μM)	DRY WT.	% INCREASE	LEVEL OF SIGNIFICANCE
10	TRIAL 1					
	-	0.0	0.0	1.19 g		
	+	1.0	0.0	1.26	+6%	N.S.
	+	3.0	1.0	1.35	+ 14	0.10
	+	7.5	1.0	1.33	+ 12	0.10
15	+	10.0	1.0	1.15	- 3	N.S.
	+ .	0.0	0.0	1.25	+ 5	N.S.
	Trial 2					
	· <del>-</del>	0.0	0.0	0.93 g		
20	+	1.0	1.0	1.06	+ 14 %	0.01
	+	5.0	1.0	1.09	+ 17	0.01
	•					
	. +	3.0	0.0	1.21	+ 30	0.01
	+	5.0	0.0	1.02	+ 10	0.05
					-	

<sup>25 \*</sup>Weights are the sum of four plants per plot (avg.) replicated 5 times. Less variation due to plant size in Trial 2 accounts for the greater level of significance of results over Trial 1.



TABLE 2. Increases in the Dry Weight of Field Corn Seedlings

Sprayed 6 Days after Germination with Formulations

of 1-Triacontanoic Acid (0.1 mg/L) and Various Metal

Ions (3 mM), at a Temperature of 26° C and pH 9.3.

Plants were Harvested 4 Days after Spraying (cv.

Pioneer 3780).

METAL ION	DRY WT.	PERCENT . INCREASES	LEVEL OF SIGNIFICANCE
CONTROL	0.87 g	<b></b> .	
Ca <sup>+2</sup>	1.14	+ 31 %	0.01
La <sup>+3</sup>	1.02	+ 17	0.01
Mg <sup>+2</sup>	1.04	+ 20	0.01
Mn <sup>+2</sup>	1.08	+ 24	0.01
Sr <sup>+2</sup>	1.10	+ 26	0.01
Pb <sup>+2</sup>	1.06	+ 22	0.01
Ba <sup>+2</sup>	1.11	+ 28	0.01
$cd^{+2}$	1.20	+ 38	0.01
. Zn <sup>+2</sup>	1.07	+ 23	0.01
Cu <sup>+2</sup>	1.07	+ 23	0.01
Co <sup>+2</sup>	1.04	+ 20	0.01

<sup>\*</sup>Weights are the sum of four plants per plot (averaged), replicated four times.



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1 TABLE 3. Increases in the Dry Weight of Field Corn Seedlings (cv. Pioneer 3780) Treated 7 Days after Germination with and without a Variety of Long-Chain Alkyl Carboxylic Acids and Esters of the Formula R-COOR' (Formulations 1, 2, & 3) and CaCl<sub>2</sub>, Harvested 4 Days after Spraying.

	<b>R</b>	R'	рH	CaCl <sub>2</sub> (mM) <sup>2</sup>	DRY WT.	% INCREASE	p*
	TRIAL 1	- Concen	trations of	f R-COOR'	= 0.1 mg	/L	
)	c <sub>24</sub>	H	9.3	3.0	1.01 g	+ 20 %	0.01
	C <sub>26</sub>	H	9.3	3.0	1.09	+ 30	0.01
	C <sub>28</sub>	H	9.3	3.0	1.10	+ 31	0.01
	c <sub>30</sub>	H	9.3	3.0	1.14	+ 36	0.01
		CH <sub>3</sub>	9.3	3.0	1.04	+ 24	0.01
;	<sup>C</sup> 36	CH <sub>3</sub>	9.3	3.0	1.09	+ 30	0.01
	c <sub>30</sub>	H	5.2	3.0	1.11	+ 32	0.01
	CONTROL	-	,		0.84		
	TRIAL 2	- Concent	tration of	R-COOR' =	0.01 mg	/L	•
	C <sub>24</sub> **	H	9.0	3.0	1.31 g	+ 25 %	0.01
}		CH <sub>3</sub>	9.0	3.0	1.36	+ 30	0.01
	C48***	CH <sub>3</sub>	9.0	3.0	1.23	+ 17	0.01
	CONTROL	-			1.05	·	

<sup>\*</sup>Level of Significance.



<sup>25 \*\*1,24-</sup>Tetracosanedicarboxylic Acid.

<sup>\*\*\*</sup>Concentration 1 µg/L.

1 TABLE 4. Increases in the Dry Weight of Field Corn Seedlings

Sprayed with Formulations of the Invention (Formulations 6 & 7) on the Seventh Day after Germination\*

and Harvested Four Days Thereafter (cv. Pioneer 3320).

5	COMPOUND	GAL/a	CONCN. (µg/L)	CaCl <sub>2</sub> (mM) <sup>2</sup>	DRY WT.	% INCR.	P
	CONTROL	<u>, , , , , , , , , , , , , , , , , , , </u>		· <b></b>	1.38 g		
	Tetracosanoic Acid	10	2	2	1.64	+19 %	0.05
	Triacontanoic Acid	10	2	2	1.60	+16	0.05
10	Beeswax	. 10	10	2	1.70	+23	0.01
	Beeswax	. 20	10	2	1.77	+28	0.01

\*All formulations were made using well water from the Northern Virginia area, pH 7.7, sprayed at  $21^{\circ}$  C.

\*\*Application rate equivalent, U.S. gallons per acre.

15 TABLE 5. Increases in the Dry Weight of Field Corn Seedlings

Sprayed with Formulations of the Invention (Formulations 6 & 7) on the Sixth Day after Germination\*

and Harvested Five Days Thereafter (cv. Pioneer 3382).

20	CONTROL	CONCN. (µg/L)	CaCl 2 (mM)	TEMP.	DRY WT.	% INCR.	Р
	CONTROL				1.20 g		
	Tetracosanoic Acid	2	2	21°	1.33	+11 %	N.S.
	Tetracosanoic Acid	2 .	2	26 <sup>0</sup>	1.51	+26	0.01
•	Beeswax	10	2	210	1.60	+33	0.01
25	Beeswax	10	2	26 <sup>0</sup>	1.41	+18	0.05

\*All formulations were made using well water from the Northern Virginia area, pH 7.7, 10 U.S. gallons/acre application rate equivalent.



1 TABLE 6. Increases in the Dry Weight of Field Corn Seedlings
(cv. Pioneer 3572) Sprayed with Various Formulations of the Invention (Formulations 6 & 7), Using
Well Water at pH 7.7, and Sprayed at an Application
Rate of 10 U.S. Gallons/Acre at Two Different
Temperatures.

concn.	CaCl <sub>2</sub> (mM)	TEMP.	DRY WT.	% INCR.	p
			1.39 g		
2	2	19 <sup>0</sup>	1.47	+ 6 %	N.S.
2	2	26 <sup>0</sup>	1.42	+ 2	N.S.
10	2	19 <sup>0</sup>	1.83	+32	0.05
10	2	26 <sup>0</sup>	1.54	+11	N.S.
	(µg/L) 2 2 10	(µg/L) (mM) <sup>2</sup> 2 2 2 2 10 2	(µg/L) (mM) ( C)  2 2 19° 2 2 26° 10 2 19°	(μg/L) (mM) <sup>2</sup> (°C) (g)  1.39 g  2 2 19° 1.47  2 2 26° 1.42  10 2 19° 1.83	(μg/L) (mM) ( C) (g) INCK.  1.39 g  2 2 19° 1.47 + 6 %  2 2 26° 1.42 + 2  10 2 19° 1.83 +32

<sup>&</sup>lt;sup>a</sup>Well water was obtained from the Northern Virginia area.

15 <sup>b</sup>Applied the 7th day after germination, harvested 4 days thereafter.



TABLE 7. Increases in the Dry Weight of Field Corn Seedlings Sprayed with Various Formulations of the Invention (Formulations 6 & 7) on the Seventh Day after Germination Using Well Water (pH 7.7) and Technical Grade CaCl, (Sprayed at 19°C).

COMPOUND	concn.	CaCl '(mM)	APPLN RATE	DRY WT.	% INCR.	р
ev. PIONEER 3744						
CONTROL			-	1.11 g		
Tetracosanoic Acid	8	2	10 g/a	1.31	+18 %	0.01
Tetracosanoic Acid	8	2	20	1.28	+15	0.01
Beeswax	10	2	10	1.35	+22	0.01
Beeswax	10	2	20	1.27	+14	0.01
cv. PIONEER 3535						
CONTROL	<del></del>		· -	1.36 g		
Tetracosanoic Acid	8	2	10	1.41	+ 4 %	N.S.
Triacontanoic Acid	1	3	20	1.40	+ 3	N.S.
Beeswax	10	2	10	1.57	+15	0.01
Beeswax	10	2	2.0	1.53	+13	0.05

<sup>20 &</sup>lt;sup>a</sup>Obtained from the Northern Virginia area.



bEquivalent application rate, in U.S. gallons per acre.

1 TABLE 8. Increases in the Dry Weight of Field Corn Seedlings (cv. Pioneer 3535) Sprayed with Formulations of the Invention Containing Beeswax (10 µg/L) and CaCl<sub>2</sub> (2 mM) at Varying Stages of Development (pH 7.4, Temperature 21.2° C, Equivalent Application Rate 10 U.S. Gal/a).

STAGES OF DEVELOPMENT	HEIGHT (cm)*	INCR. IN HEIGHT	DRY WT. (g)*	INCR. IN DRY WT.
3 Leaves	123 cm	+ 6 %	30.4 g	+ 14 %
4 Leaves	122	+ 5	30.1	+ 13
5 Leaves	124	+ 7	33.2	+ 25
6 Leaves	115	- 1	34.3	+ 29
7 Leaves	123	+ 6	26.5	0
CONTROL	116		26.6	<b></b> .

<sup>15 \*</sup>Weights are the average of 5 plots per treatment, each containing 4 plants.



1 TABLE 9. Increases in the Dry Weights of Field Corn Seedlings (cv. Beck 65X) Sprayed with Formulations 6 and 7 of the Present Invention on the 8th Day after Germination at 19°C (Application Rate Equivalent to 10 U.S. Gallons per Acre), and Harvested the 12th Day after Germination (5 Replications, 4 Plants per Plot).

	COMPOUND	concn. (pg/L)	CaCl <sub>2</sub> (mM)	DRY WT.	% INCR.	p
10	CONTROL		-	0.95 g		
	Tetracosanoic Acid	2	2	1.21	+27 %	0.01
	Beeswax	10	2	1.24	+31	0.01
	Beeswax*	10	2	1.27	+34	0.01
			•	•		

<sup>\*</sup>Two Sprayings were applied, 0.5 hr. apart.



TABLE 10. Increases in the Dry Weight of Field Corn Seedlings (cv. DeKalb XL-61) Sprayed with Various Compositions of the Invention on Day 7 after Germination at Two Application Rates, and Harvested Day 4 There
after (Sprayed at 19°C).

COMPOUND <sup>a</sup>	CONCN. (µg/L)	CaCl (mM) <sup>2</sup>	DRY WT. (g)	% INCR.	p
CONTROL			1.08 g		
Tetracosanoic Acid <sup>b</sup>	2	2	1.40	+ 30 %	0.01
Tetracosanoic Acid <sup>c</sup>	2	2	1.27	+ 18	0.05
Beeswax	10	2	1.34	+ 24	0.05
Beeswax	10	2	1.13	+ 5	N.S.

<sup>&</sup>lt;sup>a</sup>Well water from the Northern Virginia area was used in all treatments (pH 7.7).



<sup>15</sup> Applied at 10 U.S. gallons per acre equivalent.

CApplied at 20 U.S. gallons per acre equivalent.

1 TABLE 11. Increases in the Dry Weight of Field Corn Seedlings (cv. Trojan T-1000) Sprayed with Formulations of the Invention (Formulations 3, 6 & 7) at pH 7.5 and 19.5° C on Day 12 after Germination and Harvested Day 17 Thereafter.

COMPOUND	concn. (µg/L)	CaCl <sub>2</sub> (mM)	DRY WT.a	PERCENT INCREASES
CONTROL			1.98 g	
l-Tetracosanyl Tetracosanoate	1	2	2.38	+ 20 %
1-Triacontanyl Tetracosanoate	1	2	2.07	+ 5
Nervonicb	1	2	2.10	+ 6
Beeswax	10	2	2.11	+ 7
Beeswax	10	2	2.34	+ 18

<sup>&</sup>lt;sup>a</sup>Weights are the sum of 4 plants per plot, averaged for 5 replications.



bcis-15-Tetracosenoic acid.

<sup>&</sup>lt;sup>c</sup>Sprayed at 20.5° C.

1 TABLE 12. Increases in the Dry Weight of Field Corn Seedlings (cv. Trojan T-950) Sprayed with Formulations of the Invention 8 Days after Germination (tap water, pH 7.3, 10 U.S. Gal/a Application Rate Equivalent), and Harvested 4 Days Thereafter (Formulations 6 & 7).

COMPOUND	CONCN. (µg/L)	CaCl (mM) <sup>2</sup>	DRY WT. (g)	% INCR. P
CONTROL			1.13 g	
Tetracosanoic Acid	2	2	1.29	+14 % 0.0
Hexacosanoic Acid	2	2	1.23	+ 9 N.S
Beeswax .	10	2	1.41	+25 0.0
Beeswax*	· 10	2	1.25	+11 0.1

<sup>\*</sup>Sprayed with a water-soluble 15-30-15 fertilizer, 2.5 g/L.

TABLE 13 Increases in the Dry Weight of Field Corn Seedlings

(cv. Trojan T-1100) Sprayed with Formulations of the
Invention (Formulation 6) 12 Days after Germination (10 U.S. Gal/a Equivalent), Harvested 4 Days
Thereafter.

20	COMPOUND (TEMP.)	CONCN. (µg/L)	CaCl <sub>2</sub>	DRY WT.	% INCR.	p
	CONTROL			2.14 g		
	Beeswax (22°C)	10	2	2.19	+ 2 %	N.S.
	Beeswax (25°C)	. 10	2	2.33	+ 9	0.05
	Beeswax (28°C)	10	2	2.50	+ 17	0.01
25	Beeswax (22°C)	10	2	2.37	+ 11	0.05
	Beeswax (22°C)	10	2	2.27	+ 6	N.S.

<sup>&</sup>lt;sup>a</sup>Applied with urea (1 g/L) and  $ZnCl_2$  (50 mg/L).



bApplied with Banvel D (0.15 g/L).

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1 TABLE 14. Increases in the Dry Weight of Field Corn Seedlings
(cv. Pioneer 3535) Sprayed with Various Formulations
of Compounds of the Invention (0.01 mg/L) and with
1-Triacontanol (0.1 mg/L, Prior Art) on Day 11
after Germination at an Equivalent Application Rate
of 10 gal/a. Plants were Harvested 4 Days after
Spraying (4 Plants/Plot, 5 Replications).

COMPOUND	CaCl <sub>2</sub> (mM)	NAA (Μη)	pН	TEMP.	DRY WT.	% INCR.	р
CONTROL	0.0	-	_		2.13 8	<b>3</b>	
None	2.0	-	7.3	21.5°	2.19	+ 3 %	N.S.
Triacontanol ·	· 2 · 0.	-	7.3	21.5	2.12	0	N.S.
None .	6.0	1.0	8.8	21.5	2.16	+ 1	N.S.
Triacontanol	6.0	1.0	8.8	21.5	2.42	+14	0.01
Triacontanol	6.0	1.0	7.3	21.5	2.14	0	N.S.
l-Triacontanyl Tetracosanoate	2.0	-	7.3	21.5	2.40	+13	0.01
Beeswax	2.0		7.3	19.0	2.44	+15	0.01
Beeswax	2.0	-	7.3	21.5	2.35	+10	0.05
Beeswax	2.0	-	7.3	23.5	2.34	+10	0.05

Note: Formulations 1, 2, and 3 were used for all treatments except Beeswax, where Formulation 7 was used.



1 TABLE 15. Increases in the Marketable Yield of Field Corn
Treated with Formulations of the Invention Containing
10 μg/L Beeswax and 2 mM CaCl<sub>2</sub> under Various
Spraying Conditions<sup>a</sup>.

TREATED	LEAF STAGE <sup>b</sup>	TEMPERATURE	WEIGHTC	PERCENT INCREASES
cv. Trojar	n TXS 94			
-	. , <del>-</del> .		1755 g	***
+	.4	28° C	2330	+ 33 % <sup>d</sup>
cv. Pione	er 3535 <sup>e</sup>	•	•	,
-	-		4225 g	
+	3	24° C	4535	· + 7 %
+	<b>-5</b>	28 <sup>0</sup>	4500	+ 7
+	7	21°	4860	+ 15 <sup>f</sup>

<sup>15 &</sup>lt;sup>a</sup>All treatments were replicated five times. Weights adjusted to 15.5% moisture content.



bNumber of true leaves when plants were sprayed.

CWeights of grain.

dSignificant at 1% level.

<sup>20 &</sup>lt;sup>e</sup>This cultivar responds the least well of any other cultivar tested in greenhouse trials.

fSignificant at 9% level.

1 TABLE 16. Increases in the Dry Weight of Soybean Seedlings (cv. Williams) Sprayed with Formulations of the Invention (Formulations 1, 2, & 6) on Day 16 at pH 9.0 and 26° C (TRIAL 1) and on Day 16 at pH 9.5 and 27° C (TRIAL 2), Harvested 6 Days (TRIAL 1) and 4 Days (TRIAL 2) Thereafter.

COMPOUND	concn. (µg/L)	CaCl <sub>2</sub> (mM)	DRY WT.	% INCR.	<b>p</b>
TRIAL 1					
CONTROL			1.56 g		
PRIOR ART <sup>b, c</sup>	100	5	1.46	- 6 %	N.S.
PRIOR ART <sup>b,d</sup>	100	5	1.96	+ 26 %	0.10
PRIOR ART <sup>b,c</sup>	100	10	1.62	+ 4	n.s.
Triacontanoic Acid <sup>C</sup>	100	5	1.80	+ 15	N.S.
Triacontanoic Acid <sup>c</sup>	100	10	2.06	+ 32	0.05
TRIAL 2					
CONTROL	<del></del>		1.40		
PRIOR ART <sup>b,d</sup>	100	10	1.82	+ 30	0.01
Tetracosanoic Acid <sup>c</sup>	. 1	10	1.86	+ 33	0.01
Triacontanoic Acid <sup>d</sup>	10	10	1.57	+ 12	N.S.
Triacontanoic Acid <sup>C</sup>	10	10	1.83	+ 31	0.01
Triacontanoic Acid <sup>c</sup>	10	12.5	1.74	+ 24	0.01
Triacontanoic Acid <sup>c</sup>	10	15	1.83	+ 31	0.01

<sup>&</sup>lt;sup>a</sup>Weights are the sum of 4 plants per plot averaged, replicated 5 times.

<sup>30</sup> dSeedlings were fertilized before spraying with a water-soluble 15-30-15 fertilizer (2.5 g/L, 50 mL per plot).



bCompound = 1-triacontanol, in accordance with U.S. Patent 4,333,758 to the present inventor.

<sup>&</sup>lt;sup>c</sup>Seedlings were fertilized 1 day <u>after</u> spraying with a water-soluble 15-30-15 fertilizer (2.5 g/L, 50 mL per plot).

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1 TABLE 17. Increases in the Dry Weight of Soybean Seedlings (cv. Williams) Sprayed with Formulations of the Invention Containing Compounds of the Structure R-COOR' (Formulations 3, 4, 6 & 8, 10 µg/L) and Ca<sup>+2</sup> (10 mM) at Varying Stages of Development and Temperatures (pH 7.4 to 7.6).

R	R'	STAGE	TEMP.	DAY <sup>b</sup>	DRY WT.C	% INCREASE
CONTE	OL				7.07 g	<u>-</u>
Beesv	ax d	2 sets	24° C	16	9.49	+ 34 %
Beesv	ax d	4 sets	24 <sup>0</sup>	28	8.95	+ 27
Beesv	<sub>zax</sub> d	3 sets	33°.	22	8.58	+ 21
c <sub>24</sub> e	H	3 sets	28°	22	7.61	+ 8
c <sub>24</sub>	H	3 sets	28°	22	8.53	+ 21
c <sub>24</sub>	c <sub>24</sub>	3 sets	28 <sup>0</sup>	22	9.30	+ 32
c <sub>24</sub>	c <sub>30</sub>	3 sets	28°	22	7.63	+ 8

The number of sets of leaves refers to the number of sets of 3 leaves each(trifoliate stage).

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Numbers of days after germination upon which each treatment was sprayed.

<sup>&</sup>lt;sup>c</sup>Weights are the sum of 4 plants per plot, replicated 5 times.  $^{d}$ Mixture containing compounds wherein R =  $^{2}$ 4 to 36 and R' =  $^{2}$ 4 to 36, see text.

eNervonic acid (cis-15-tetracosenoic acid).

1 TABLE 18. Increases in the Marketable Yield of Soybeans (cv. Williams) Sprayed with Formulations of the Invention at the Trifoliate Stage at 30°C and pH 7.6°a (Formulations 6 & 8).

COMPOUND	CONCN. (µg/L)	CaCl <sub>2</sub> (mM)	WEIGHT <sup>b</sup>	% INCR.	р
CONTROL			322.0 g		
Triacontanoic Acid	5	10	502.3	+ 56 %	0.01
Beeswax	10	10	493.3	+ 53	0.01

<sup>10</sup> aReplicated five times.



bWeights per 100 plants (6 meters combined length), grain weight adjusted to 13.5% moisture content.

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1 TABLE 19. Increases in the Dry Weight of Pea Seedlings
(cv. Early Alaska) Sprayed with Formulations of
the Invention (Formulations 6 & 8) on Day 8 after
Germination at pH 9.3 and 26° C and Harvested 4
Days Thereafter.

COMPOUND	concn. (µg/L)	CaCl <sub>2</sub> (mM)	DRY WT.	% INCR.	p
CONTROL :	_ `. `		1.11 g		
Triacontanoic Acid	100	10	1.29	+ 16 %	0.01
Triacontanoic Acid	100.	15	1.32	+ 19	0.01
Triacontanoic Acid	1	15	1.26	+ 14	0.01
Tetracosanoic Acid	1	15	1.42	+ 28	0.01

\*Weights are the sum of 8 plants per plot, replicated 5 times.

Plants were sprayed at an equivalent application rate of

20 U.S. gallons/acre. Weights reported are averages/5 plots.

TABLE 20. Increases in Dry Weight of Pea Seedlings (cv. Sugar Snap) Sprayed with Formulations of the Invention (Formulation 6 & 8) on Day 7 after Germination at pH 9.0 and 26°C and Harvested 7 Days Thereafter.

COMPOUND	concn. (µg/L)	CaCl <sub>2</sub> (mM)	DRY WT.	% INCR.	p
CONTROL			0.56 g		,
Tetracosanoic Acid	1	15.0	0.83	+ 48 %	0.01
Docosanoic Acid	1	15.0	0.76	+ 36	0.01
Prior Art**	100	12.5	0.69	+ 23	0.10

<sup>\*</sup> Weights are the sum of 3 plants per plot (average), replicated 5 times.

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<sup>\*\*</sup>Compound = 1-triacontanol, formulated in accordance with

U.S. Patent No. 4,333,758.

COMPOUND	CONCN. (µg/L)	CaCl <sub>2</sub> (mM)	DRY WT.a	% INCR.	p
cv. EARLY ALASKA	:3f2				<u>.</u>
CONTROL			54.0 g	:	
Tetracosanoic Acid	15	15	78.4	+45 %	0.05
Beeswax	15	15	84.1	+56	0.05
Beeswax	- 15	15	81.4	+51	0:05
cv. SUGAR SNAP					
CONTROL .			164.3 g		
Prior Art <sup>c</sup>	100	12.5	184.7	+12	N.S.
Docosanoic Acid	. 2	15	235.9	+44	0.05
Tetracosanoic Acid	2	15	234.5	+43	0.05
Beeswax	10	15	218.8	+33	0.10

<sup>20 &</sup>lt;sup>a</sup>Weight per 8 plants/plot, averaged for 4 replications.



b<sub>2</sub> sprayings, 1 hr. apart.

<sup>&</sup>lt;sup>c</sup>Compound = 1-triacontanol, pH adjusted to 10.1. This cv. was found to respond poorly to optimum formulations of triacontanol.

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1 TABLE 22. Increases in the Dry Weight of Tomato Seedlings

Sprayed with Formulations of the Invention

(Formulations 1, 2, 6 & 8) when Plant Had 3 Pairs\*

of True Leaves, Harvested 4 Days Thereafter.

COMPOUND	concn. (µg/L)	CaCl <sub>2</sub> (mM)	pH -	DRY WT.	% INCR.	Р
TRIAL 1: cv. Campbe	11's 132	<u>7</u>	<del></del>			
CONTROL			- <b></b>	1.62 g		
Tetracosanoic Acid	74 <u>241</u>	10	8.2	1.84	+ 14 %	n.s.
None		10	8.2	1.62	0	N.S.
Efcosanoic Acid	1	10	8.2	2.35	+ 45	0.05
Tetracosanoic Acid	3	10	6.8	1.84	+ 14	N.S.
Beeswax	1	10	8.2	1.55	- 4	N.S.
Beeswax	10	10.	8.2	1.95	+ 20	0.10
Beeswax	100	10	8.2	2.24	+ 38	0.05
Beeswax	.10	10 ·	6.8	2.03	+ 25	N.S. 0.05 N.S. N.S. 0.10 0.05
Docosanoic Acid	1	10	8.2	2.19	+ 35	0.05
TRIAL 2: cv. Red Ch	erry	•	•			
CONTROL				2.66		
Tetradecanoic Acid	10	10	7.3	2.30	- 14	N.S.
Hexadecanoic Acid	10	10	7.3	3.18	+ 20	0.05
Oleic Acid	10	10	7.3	2.99	+ 12	N.S.
Eicosanoic Acid	. 10	10	7.3	3.07	+ 15	0.10

<sup>25 \*</sup>cv. Red Cherry was sprayed when plants had between 5 and 6 sets of true leaves, some of which were budding.



<sup>\*\*</sup>Weights are the average of plots having 4 plants each, replicated 4 times (cv. Campbell's 1327) or 5 times (cv. Red Cherry). All plants were sprayed between 26° and 28° C.

TABLE 23. Increases in the Marketable Yield of Tomatoes

Sprayed with Formulations 6 and 8 of the Invention

when Seedlings Had Four Sets of True Leaves

(pH 7.4, Temp. = 30° C, cv. Better Boy)

5	COMPOUND	CONCN. (µg/L)	CaCl <sub>2</sub> (mM)	FRESH WEIGHT	PERCENT INCREASE
	CONTROL	_		32435 g	
~	Tetracosanoic Acid	2	10	53082	+ 64 %
-	Beeswax	10	10	43266	, + 33
10	Beeswax*	10	10	43700	* + 35

<sup>\*</sup>Two treatments, one hour apart.

Note: Treatments were made at an application rate equivalent to 20 U.S. gallons/acre.



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1 TABLE 24. Increases in the Dry Weight of Wheat Seedlings
(cv. Potomac) Sprayed with Formulations of the
Invention (Formulations 6 & 8) when Seedlings Had
3 to 4 True Leaves (pH 7.5), Harvested 8 Days
Thereafter.

	COMPOUND	CONCN. (µg/L)	CaCl <sub>2</sub> (mM)	TEMP.	DRY WT.	% INCR.	p į
	CONTROL	<b></b>			1.124 g	·	
	Tetracosanoic Acid	20	20	20 <sup>0</sup> C	1.087	- 3 %	N.S.
10	Beeswax	10	10	20°	1.164	+ 4	N.S.
	Beeswax	20	20	20 <sup>0</sup>	1.554	+38	0.01
	Beeswax	. 10	10	27 <sup>0</sup>	1.111	<b>- 1</b>	N.S.
	PRIOR ART**	100	20	20 <sup>0</sup>	1.197	+ 6	N.S.
	PRIOR ART	100	20	26 <sup>0</sup>	1.371	+22	0.01
15	PRIOR ART	100	15	26 <sup>0</sup>	1.248	+11	N.S.
	PRIOR ART	100	25	26 <sup>0</sup>	1.461	+30	0.01

<sup>\*</sup>Weights are the averages of plots having 6 plants each, replicated 6 times.



<sup>\*\*</sup>PRIOR ART formulation tested contained 100 µg/L 1-triacontanol
(U.S. Patent No. 4,333,758 to the present inventor), pH 9.5
to 9.9.

1 TABLE 25. Increases in the Dry Weight of Wheat Seedlings
Sprayed with Formulations of the Invention
(Formulation 7) at 20°C and pH 7.5 when Seedlings
Had about 4 True Leaves, Harvested 5 Days Thereafter.
Plants Were Subjected to Drought Conditions (Air
Circulated 6 hr./day at 36.5°C and 52% Relative
Humidity). Applied at 10 U.S. Gal/a Equivalent.

	COMPOUND	concn. (μg/L)	CaCl <sub>2</sub> (mM)	DRY WT.	% INCR.	WATER CONTENT	% INCR.
10	CONTROL			2.759 g		11.18 g	
	Beeswax	1.5	15	3.358	+22 %	16.24	+45 %
	Beeswax	20	20	3.186	+15	#3.47	+20
	Beeswax	25	25	2.863	+ 4	13.35	+19
	Beeswaxb	20	20	2.722	+ 7 <sup>C</sup>	12.41	+ 1 <sup>c</sup>

<sup>&</sup>lt;sup>a</sup>Weights are the average of plants containing 20 plants each.

<sup>b</sup>Soil drench using 50 mL of the formulation applied to the roots of the plants (per plot).



 $<sup>^{</sup>m C}$ Increase reported over controls which were treated with 50 mL of 20 mM CaCl $_2$  solution per plot.

Note: When plants were not subjected to drought conditions, normal water content increases were in the 7 to 10 % range.

1 TABLE 26. Increases in the Dry Weight of Sweet Corn Seedlings (cv. Silver Queen) Sprayed with Formulation 7 of the Invention on Day 9 after Germination and Harvested Five Days Thereafter (Sprayed at 25° C and pH 6.8).

COMPOUND	CONCN. (pg/L)	CaCl <sub>2</sub> (mM)	DRY WT.	PERCENT INCREASES	р
CONTROL		~-	1.27 g		
Beeswax	10.0	2.0 %		+ 2 %	N.S.
Beeswax	7.5	7.5	1.48	+ 17	0.05
Beeswax	10.0	10.0	1.63	+ 28	0.01
Beeswax	12.5	12.5	1.34	+ 6	N.S.

<sup>\*</sup>Weights are the average of five plots, four plants per plot.

TABLE 27. Increases in the Marketable Yield of Sweet Corn

(cv. Golden X Bantam) Sprayed with Formulations 6

and 8 of the Invention When Seedlings Had Four

True Leaves (28° C, pH 7.3, 10 U.S. Gallons/Acre

Equivalent Application Rate)\*.

				<del></del>	
COMPOUND	concn. (µg/L)	CaCl <sub>2</sub> (mM)	WEIGHT OF EARS	PERCENT INCREASES	p
CONTROL			1822 g		
Tetracosano	oic			•	
Acid	2	10	2478	+ 36 %	0.05
Beeswax	- 10	10	2853	+ 57	0.01

<sup>25 \*</sup>Replicated three times.



1 TABLE 28. Increases in the Dry Weight of Tobacco Seedlings
(cv. Broad Leaf Hicks) Sprayed with Formulations of
the Invention (Formulations 6 & 8) on Day 52 after
Germination (22°C, pH 7.4) and Harvested Four Days
Thereafter.

	COMPOUND	٠	CONCN.	CaCl <sub>2</sub> (mM)	DRY WT.	PERCENT INCREASES	p	· ··
	CONTROL	•			0.93 g	- <del>-</del>		
	Beeswax		5	5	1.17	+ 26 %	0.05	
)	Beeswax		10	10 .	1.17	+ 26	0.05	,
	Beeswax	-	15	15	1.23	+ 32	0.01	
	Tetracosanoic A	Acid	5	. 5	1.08	+ 16	N.S.	
	Tetracosanoic A			10	1.15	+ 24	0.05	
	· · · · · · · · · · · · ·		•.•	··· ·· · · · · · · · · · · · · · · · ·				

<sup>\*</sup>Weights are for one plant per plot, replicated five times.

15 TABLE 29. Increases in the Dry Weight of Cotton Seedlings
(cv. McNair 235) Sprayed with Formulations 3, 6 & 8
of the Invention on Day 50 after Germination (3rd
to 4th Set of True Leaves) at pH 7.6 and 24 C
(20 U.S. Gallons per Acre Equivalent Application Rate).

20	COMPOUND	CONCN. (µg/L)	CaCl <sub>2</sub> (mM)	DRY WT.	PERCENT INCREASES	р
	CONTROL	<b>-</b> -		4.72 g	<b></b>	
	Tetracosanoic Acid	10	10	6.47	+ 37 %	0.01
25	1-Triacontanyl Tetracosanoate	10	10	6.17	+ 31	0.01
	Beeswax ·	10	10	6.66	+ 41	0.01

<sup>\*</sup>Weights are the average of five plots per treatment, four plants per plot.



1 TABLE 30. Increases in the Dry Weight of Pepper Seedlings

Sprayed with Compounds of the Formula R-COOH

(1 μg/L) 32 Days after Germination, and Harvested

4 Days Thereafter (6 Plants per Plot, 5 Replications).

5 R*		CaCl <sub>2</sub> (mM)	DRY WT.	PERCENT INCREASES	LEVEL OF SIGNIFICANCE	
•			1.31 g			- *
	c <sub>30</sub>	10.0	1.58	+ 21 %	0.10	
	c <sub>24</sub>	5.0	1.67	+ 27	0.05	
10	C <sub>24</sub>	10.0	1.29	- 2	n.s.	
	c <sub>24</sub>	15.0	1.42	+ 8	N.S.	

<sup>\*</sup>Formulations 1 and 2 were used. The cv. used was 'Early California Wonder'.

TABLE 31. Increases in the Marketable Yield of Peppers

(cv. Red Chili) Sprayed with Formulation 6 of the

Invention at 22° C and pH 7.2 when Plants had 4 to

5 Sets of True Leaves.

COMPOUND	CONCN. (µg/L)	CaCl (mM)	WEIGHT	% INCR.	P
CONTROL			49.8 g		
Triacontanoic Acid	. 5	10	90.8	+ 82 %	0.01

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1 TABLE 32. Increases in the Marketable Yield of Lettuce (cv. Buttercrunch) Sprayed with Formulation 7 at pH 7.5, 25° C, and 76% Relative Humidity. Plants had 4 to 5 Sets of True Leaves, and Were Harvested 1 Month after Spraying.

COMPOUND	CONCEN- TRATION	CaCl <sub>2</sub> (mM)	<del></del>	WT. PER HEAD	PERCENT INCREASES	P*
CONTROL				228 g		
Beeswax	. 10 μg/Ĺ	5	. O. O.	296	+ 30 %	0.05
Beeswax	10	10	10.0	304	+ 33	0.05

\*Level of Significance (4 Replications).

TABLE 33. Increases in the Growth of Potatoes\* (cv. Irish Cobler) Sprayed with Formulations of Various Compounds of the Invention (Formulation 7) 24 Days after Planting, Harvested 6 Days after Spraying (pH 7.6, Five Replications).

COMPOUND	CONCEN- TRATION	CaCl <sub>2</sub> (mM)	TEMP.	DRY WT.	PERCENT INCREASES
CONTROL				2.69 g	
Tetracosa- noic Acid	2 µg/L	10	21° C	3.32-	+:23 %
Beeswax	10	10	21 <sup>0</sup>	3.43	+ 28
Beeswax	10	10	240	3.06	+ 14

\*As determined by the weight of foliage.



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1 TABLE 34 Increases in the Marketable Yield of Radishes
(cv. Champion) Sprayed with Various Formulations
of the Invention (Formulations 6 & 8) at 27° C
when Plants had 3 Pairs of True Leaves (pH 6.9).
Weights are for 5 Plants per Plot, Replicated
Five Times.

	COMPOUND	CONCN.	CaCl <sub>2</sub> (mM)	WEIGHT	% INCR.	P
	CONTROL			16.1 g		
10	Tetracosanoic Acid	4	10	23.6	+ 47 %	0.05
2 -	Tetracosanoic Acid	6	<sup>;</sup> 15	15.5	- 4	N.S.
	Beeswax	10	. 10	21.8	+ 35	0.10
•	Beeswax	15	15	21.8	+ 35	0.10
				•		

<sup>\*</sup>Without foliage.

15 TABLE 35. Increases in the Growth of Kentucky Bluegrass
(Blend) Treated with Formulation 8 of the Invention
at Various Stages of Development at an Application
Rate Equivalent to 20 U.S. Gallons/Acre (29° C,
pH 7.5, Replicated Five Times).

<b>)</b> .	COMPOUND	CONCN. (µg/L)	CaCl <sub>2</sub> (mM)	HEIGHT	DRY WT.	% INCR.	_ P
	CONTROL				388 g		
	Beeswax	10	10	3.5 cm	383	<b>-</b> 1 %	N.S.
	Beeswax	10	10	6.0	487	+ 26 %	0.01
5	Beeswax	10	10	8.5	379	- 2	N.S.
	Beeswax	15	15	8.5	381	- 2	N.S.

<sup>\*</sup>Dry weight of cut grass per m<sup>2</sup> at a height of 15 cm at harvest.



1 TABLE 36. Increases in the Number of Marketable Fruit of Certain Crops Sprayed with Formulations of the Invention (Formulations 2, 3, 6, & 7) Observed Mid-Season (over Control Plots).

<b>5</b> .	CROP	CULTIVAR .,	COMPOUND *	CaCl <sub>2</sub> (mM)	% INCR:
	Field Corn	Trojan TXS-94	Beeswax .	. <b>2</b> .	+ 23 %
	Tomatoes	Better Boy	Tetracosanoic Acid	10	+ 61
	11	**	Beeswax**	10	+ 75
10	Peppers	Red Chili	Triacontanoic Acid	10	+ 30

<sup>\*</sup>Concentration = 10  $\mu$ g/L.

TABLE 37. Increases in the Dry Weights of other Seedlings
Sprayed with Formulations of the Invention

(Formulation 1) at pH 9 to 9.5 and 22° to 26° C,
Harvested 4 to 7 Days Thereafter.

	CROP	Ca <sup>+2</sup> (mM)	COM- POUND <sup>a</sup>	DRY WT.	% INCR.	Р
20	Alfalfa (cv. Kansas)	0		0.397 g		
	(0),,	10	+	0.790	+ 99 %	0.01
	Barley (cv. Barsoy)	0	-	0.545		
		10	<del>;+</del>	0.730	+ 34	0.05
	Beans	0	-	1.05		
25	•	· 5	. +	1.14	+ 9	N.S.
	,	10	+	1.53	+ 46	0.10

al-Triacontanoic acid, 0.1 mg/L for all.

3:5.

<sup>\*\*</sup>Applied twice, 1 hour between applications.

bPlants/plot:replications - alfalfa, 8:6; barley, 5:5, beans,

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1 TABLE 38. Increases in the Yield of Alfalfa (cv. Kansas)

Sprayed with Formulations of the Invention Containing
10 μg/L Beeswax and 10 mM CaCl<sub>2</sub> at pH 7.4 When

Plants Were 15 to 20 cm in Height. Plants Were

Harvested on Two Occasions When 30 cm in Height.

		•		
TREATED	: SPRAY TEMPERATURE	DRY WT. b	PERCENT INCREASES	LEVEL OF SIGNIFICANCE
First C	utting			
-		421 g		
+	24° C	606	+ 44 %	0.05
+	30° C	571	+ 36	0.05
+	33 <sup>0</sup>	501 <del>:</del>	+ 19	N.S.
Second (	CuttingC			
-	1 146 2	311 g	• .	
-	2.4° C	484	+ 56 %	0.01
+	30°	365	+ 17	N.S.
-	33°	457	+ 47	0.01

<sup>&</sup>lt;sup>a</sup>Hard water was used in all sprayings, application rate equivalent to 20 U.S. gallons per acre.



bDry weights per m<sup>2</sup> average, replicated 5 times.

Treatment of new growth after first cutting did not result in a significant increase in yield.

1 TABLE 39. Increases in the Marketable Yield of Beans (cv. Blue Lakes) and Cucumbers (cv. Straight 8) Treated with Formulation 8 of the Invention (Beeswax = 10 µg/L, CaCl<sub>2</sub> = 10 mM), Applied at an Application Rate of 20 U.S. Gallons per Acre.

CROP	TREATMENT	FRESH WT.	% INCREASE	P
Cucumber	Control	9425 g		
Cucumber	Beeswax	20640	+ 119 %	0.05
Beans	Control	840		
Beans	Beeswax	1239	+ 48	0.10



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## EXAMPLE XI: FIELD CORN

An in depth study of the effects of the compositions of the present invention was made using various field corn cultivars, since field corn is the major agricultural commodity in the U.S. Table 1 shows the results obtained in promoting the growth of field corn seedlings (cv. Pioneer 3780) using various formulations of the invention, and further demonstates how additional plant growth substances may be used to enlarge the effective range of metal ion concentrations which are effective in the compositions to stimulate corn growth. Usually, the addition of such substances may not be required in the formulations due to the broad range of effective concentrations of metal ions on field corn (between about 1.5 and 4 mM being preferred). Fig. 1 further shows the extremely low concentrations of one compound, 1-triacontanoic acid, which are effective in stimulating plant growth, and also the broad range of concentrations which may be used while maintaining an high level of growth-stimulating activity in corn seedlings.

While Ca<sup>+2</sup> is the preferred metal ion of the invention due to low cost and other factors hereinabove described, Table 2 shows the efficacy of a large number of metal ions having a valence of +2 or more in the formulations of the invention. The data here clearly indicate that all polyvalent metal ions are useful in the compositions to stimulate plant growth. While some metal ions, such as Cd<sup>+2</sup>, are somewhat more effective than others, their use may be expected to be more limited and the relatively high toxicity, and the use of said metal ions may best be limited to plant life not used for food purposes and the like.

Table 3 shows the effectiveness of a broad range of compounds of the invention in combination with Ca<sup>+2</sup> on field corn (cv. Pioneer 3780), ranging from 24 to 48 carbon atoms in chain lengths of the carboxylic acids or their esters.

35 Also, the pH of the solution applied to the corn seedlings

BUREAU OMPI WIPO WIPO appears to have no influence on the results obtained, with similar increases in dry weight being observed for formulations at pH 9.3 and 5.2.

Tables 4 and 5 show additional data for field corn

(cvs. Pioneer 3320 and 3382, respectively), and include the mixture of esters contained in beeswax. In these and other examples, it was found that the use of technical grade CaCl<sub>2</sub> did not affect the results obtained with the formulations, nor did the use of hard water. This indicates that Formulations 6 & 7 of the invention are highly economical due to the low cost of both beeswax and technical grade CaCl<sub>2</sub>. The CaCl<sub>2</sub> used is further known to contain about 77% CaCl<sub>2</sub> with the balance being other metal ion salts, some of which are those of the present invention.

Table 6 shows the effects of temperature on cv. Pioneer 3572 when treated with various formulations, and, surprisingly, indicates that lower temperatures of application for field corn cvs. may be preferred. Since field corn is generally sprayed with the formulations of the invention at an early stage, and since spraying is usually accomplished by growers either early in the morning or in the evening when temperatures are cooler, this would appear to be of advantage.

Table 7 compares application rates of the formulations of the invention on cvs. Pioneer 3744 and 3535. The data therein indicate that lower application rates may be preferred over higher ones, thereby making application of the formulations of the invention even more economical. Pioneer 3535 responds relatively poorly than other cultivars tested, and this may be related to the rapid growth rate observed with this cultivar. However, Table 8 shows that the stage at which the cultivar is sprayed influences the results, and other cultivars may be expected to respond to a higher degree when sprayed at different stages.



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Beck 65X, and further shows that no additional effect was observed with additional sprayings. It has been observed, however, that corn plants sprayed in the field with multiple sprayings show the appearance of an additional ear per plant not observed on control plants. While this effect may depend on the variety of corn used, it has been observed with single sprayings on cv. Trojan TXS-94, but not on cv. Pioneer 3535.

Tables 10 and 11 show additional data obtained on cvs. DeKalb XL-61 and Trojan T-1000. Further support is given in Table 10 for the use of lower application rates, and Table 11 shows the efficacy of long-chain esters of the formula R-COOR', wherein R = 24 carbon atoms and R' = 24and 30 carbon atoms. A reduced effect is observed when R' increases in length for this variety of field corn, and in general, the use of the waxy esters shows improved results over the use of the free acids. Also, nervonic acid, a 24carbon unsaturated acid (cis-15-tetracosenoic acid) shows some effect which is similar to that observed under the same conditions when beeswax was applied in the formulations of the invention. It is interesting to note that this cultivar (T-1000) and other later maturing cultivars respond better at a relatively higher temperature than the cultivars mentioned hitherto. Since these cultivars require a longer . time to approach a stage where spraying should be done, this may be of advantage since temperatures at which the cultivars should be sprayed would be higher at that point in the growing season, and further advantage may be presented thereby.



Tables 12 and 13 show that formulations of the invention 1 show compatibility with fertilizers and herbicides on cvs. Trojan T-950 and Trojan T-1100, respectively. While high phosphate fertilizers may precipitate the metal ions of the invention in aqueous solution, effects on increasing 5 the growth of corn seedlings are nevertheless noted. cv. T-1100 requires a high temperature for activity when sprayed with the formulations relative to other cultivars tested, an improved result, which shows statistical significance, is observed with the inclusion of a urea/Zn foliar 10 fertilizer in the formulation even at the lower temperature. The addition of the herbicide Banvel D also shows a slightly improved result, however, this was not found statistically This indicates that the formulations of the significant. present invention are useful when combined with agricultural 15 products in common use, thereby reducing additional labor costs which would be incurred by a separate, additional spraying of the formulations.

Table 14 compares the results observed using the compositions of the present invention containing beeswax and 20 1-triacontanyl tetracosanoate and those of the prior art (as described in U.S. Patent No. 4,333,758 to the present inventor, and related applications). In the prior art, 1-triacontanol, which is also contained in beeswax in small quantities, is found to stimulate plant growth only at 25 alkaline pH, as shown for cv. Pioneer 3535. Table 14 ' shows that no growth stimulation is observed with metal ions alone in solution, and that the triacontanol formulation containing NAA is effective only at alkaline pH. results are observed when triacontanol is applied to plant 30 life using the formulations of the present invention which contain a metal ion concentration of 2 mM at pH 7.3, said triacontanol being present at a concentration of 0.1 mg/L, i.e., ten time that of the beeswax present in solution in the formulations used under the same conditions. 35

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Also, 1-triacontanyl tetracosanoate, an ester of tetracosanoic acid also present in beeswax, is highly effective in stimulating plant growth in the formulation used herein, indicating that the carboxylic acid moiety is required for the activity of the compounds of the invention, thereby clearly distinguishing the invention from the prior art.

Field trials conducted with field corn showed positive results on a number of cultivars tested, two of which are shown in Table 15. The Trojan TXS 94 cultivar showed typical results found with other cultivars, and cv. Pioneer 3535, a cultivar which was found to respond relatively poorly to other cultivars tested, showed somewhat lesser increases. Upon examining the data for this cultivar, however, it is observed that the conditions used in field trials differed from those recommended by short-term greenhouse trials (see Table 7) in that the formulation containing beeswax was found to be more effective at a lower spraying temperature than the temperature at which the formulations were sprayed during field trials. In view of this observation, it may be concluded that field corn responds more favorably when plants have developed farther than the three-leaf stage, but this is not necessary for activity. An additional observation of interest is that this cultivar of field corn responds to formulations of the invention up to about the seven-leaf stage in greenhouse trials, at which stage activity is observed to diminish (Fig. 3). In field trials, however, increases in crop yield are noted even when seedlings were sprayed at the seven-leaf stage (Table 15). This may be due, however, to the fact that plants treated in greenhouse-. controlled environments with the formulations of the present invention are grown in pots, thereby causing limited volume for root growth to be a contributing factor.

It is therefore apparent that positive results are achieved through the application of the formulations of the invention under a variety of conditions and stages of plant

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growth, however, by altering the conditions under which the formulations of the invention are applied, one may improve the results observed therefrom.

EXAMPLE XII: SOYBEANS

As shown in Table 16, soybean seedlings respond well to formulations of the present invention at preferred metal ion concentrations between about 5 and 15 mM. When the results thus achieved are compared to formulations found in the prior art, as more fully described in the foregoing discussion for field corn, the present invention is distinguished from the prior art by the fact that soybean seedlings have been found to respond to formulations of triacontanol only when the soil in which they are grown has sufficiently high phosphate From these results, it becomes apparent to one skilled in the art that the growth-stimulating activity of the formulations of the present invention that contain beeswax cannot be attributed to traces of 1-triacontanol contained therein, or in other naturally-occurring waxes (see Table 17). It is further observed that the naturally-occurring constituents that are compounds of the present invention, especially waxy esters, are highly active in promoting the growth of soybean seedlings, and unsaturated acids are also effective in the formulations of the invention containing metal ions.

The stage at which seedlings were treated somewhat alters the response obtained, with a stage between about the second and fourth trifoliate stage being preferred. Also, the temperature at which soybeans are preferably sprayed or otherwise treated with formulations of the invention appears to be broader than that observed in the case of field corn seedlings. Similar results are found using other cultivars of soybeans, such as cv. Essex and the like.

As in the case of other crops, it is also observed that soybeans respond preferably to straight-chain carboxylic acids or salts or derivatives thereof which have a relatively



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longer hydrocarbon chain length than other crops such as tomatoes, peas and the like, i.e., compounds having about 30 carbon atoms produce a better response when combined in the formulations of the present invention than compounds of the invention having only about 24 carbon atoms or less. For example, field corn appears to respond to a relatively longer hydrocarbon chain length in the formulations than tomatoes (see Fig. 2).

Increases in crop yield of soybeans has been observed (Table 18) using cv. Williams and other cultivars. Soybeans, furthermore, appear to show a rapid increase in growth in field trials, as do many other crops, shortly after application of the formulations of the present invention.

## EXAMPLE XIII: PEAS

Peas were tested with the formulations of the present invention with favorable results, both in greenhouse trials and in the field. A comparison of the greenhouse data presented in Tables 19 and 20 with the actual field test data obtained and shown in Table 21 shows that increases in the field promoted by the application of the formulations may be expected to equal or exceed those observed in the short-term greenhouse trials. It is therefore clear that the procedures used in these greenhouse trials at an early stage of plant development present a method highly useful in both the prediction of optimum formulations for use in field trials, and in predicting what minimum increase in crop yield may be expected through the application of the optimum formulations.

Unlike soybeans, peas respond to formulations of the invention to a higher degree when the hydrocarbon chain length of the compounds contained therein is relatively



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and in the field.

short, i.e., 22 or 24 carbon atoms as compared to about 30 carbon atoms. Also the formulations of the present invention are found superior in stimulating the growth of pea cultivars which respond somewhat poorly to triacontanol formulations, such as cv. Sugar Snap (prior art), both in the greenhouse 5

In the case of peas, it may be desirable to use other more economical, naturally-occurring waxes, oils, and the like which contain a lower average molecular weight hydrocarbon chain, such as peanut oil (C16 to C24), jojoba oil  $(C_{20}$  to  $C_{22}$ ), and other such waxes and oils and the like which are obtained from plant and animal sources. Other compounds within the scope of the present invention which show increases in the growth of peas and may be expected to to show increases in the growth of other plant life when used in accordance with the invention also include, but are not limited to, erucic acid, tribehenin, dibehenoyl phosphatidyl choline (or other acidic phospholipids), and the like, with lower responses being observed for compounds such as 20 4,7,10,13,16,19-docosahexaenoic acid, 2-hydroxydocosanoic acid, lecithin, etc.

## EXAMPLE XIV: TOMATOES

Several cultivars of tomatoes were treated with the formulations of the present invention with similar increases in growth resulting therefrom. The data for the two cultivars shown in Table 22 indicate that tomatoes respond to formulations of the invention containing compounds of the formula R-COOR', wherein R is between about 16 and 36 carbon atoms in length, with a relatively shorter hydrocarbon chain length being preferred to that in the case of The more preferred hydrocarbon chain lengths are those where R = 16 to about 24 carbon atoms, with 20 carbons being most preferred (Fig. 2). As a result, many naturally-occurring waxes, oils, and the like are



useful in the formulations of the present invention, such as beeswax ( $C_{24}$  to  $C_{36}$ ), jojoba wax or oil ( $C_{20}$  to  $C_{22}$ ), olive oil ( $C_{16}$  to  $C_{20}$ ), rice bran oil ( $C_{16}$  to  $C_{18}$ ), soybean oil, and the like. In fact, even human sebum may be expected to have an effect on plant life when combined in the formulations of the present invention, causing increases in the growth of plant life.

Some differences between tomato cultivars is apparent in their response to the compositions of the instant invention, as shown for the two cultivars in Table 22 in their response to formulations containing eicosanoic acid  $(C_{20})$ . While increases in plant growth are seen either with saturated or unsaturated compounds in the formulations, when the hydrocarbon chain is lowered to about 14 carbon atoms, a considerable decrease in plant growth is observed. 15 Since the prior art teaches that such compounds are inhibitory in their effects on plant growth as described more fully in the foregoing background of the prior art when applied without the addition of the metal ions of the present invention, including the 14-carbon analog and higher analogs, 20 the surprising transition between growth inhibition and growth stimulation observed through the use of the formulations of the invention containing these compounds is not clear as to the possible mechanism whereby the compositions herein achieve their results. It may also be possible that 25 compositions such as those described herein containing relatively lower molecular weight compounds (e.g., wherein R = 14 carbon atoms or less) which are known to cause growth inhibition, may be superior in their effects in decreasing the growth of plant life when combined with 30 metal ions of the present invention.

Preliminary observations during field trials on tomatoes has shown that large increases in the number of tomatoes occurs on plants sprayed with the formulations of the present invention. As a result of field trials,

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marketable increases in yield are found to be similar to those increases in dry weight observed in the greenhouse trials (see Tables 22 and 23). Other cultivars have been found to show increases in yield, e.g., cv. Campbells 1327 gave 20 to 34% higher yields compared to controls, and cv. Ace 55 showed increases up to about 31%.

Also, in addition to increases observed when tomato seedlings were sprayed with formulations of the present invention, increases in the dry weight of seedlings grown from seed soaked in a formulation containing beeswax (10  $\mu g/L$ ) and Ca+2 (10 mM) for about 1.5 hours prior to planting were observed to be 33% over control plants grown from untreated seed (p = 0.01) when plants reached a stage where they had four sets of true leaves, This result indicates that the compositions of the invention are highly effective in improving the quality of seed prior to planting, and this mode of application may be preferred for seed of various crops which require long periods of time to germinate and produce seedlings of suitable size for transplanting in the field, such as tomatoes, tobacco, celery, lettuce, wheat, rice, and the like. Subsequent application of the formulations as foliar sprays, soil drenches, and the like, may be expected to further improve the growth of. plant life and yields of certain crops.



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## EXAMPLE XV: WHEAT

Winter wheat was tested with the formulations of the present invention under different conditions, and found to respond comparably to field corn with respect to increases in dry weight observed. However, it was noted that the concentrations of metal ions employed in the formulations for an optimum effect was somewhat higher than that observed for most other crops and plant life, and similar to that observed for peas.

10 Table 21 shows the range of metal ion concentrations effective in the formulations for winter wheat (cv. Potomac), and compares them with those found useful in the prior art. It is observed that the temperatures at which a response is noted when the formulations are applied to wheat seedlings is significantly lower than that whereby the prior art 15 shows an effect in plant growth stimulation. Also, the metal ion concentration effective is lower than that required for optimum activity using prior art formulations. presents two advantages: (1) the cost of formulating the 20 product is reduced, and (2) the temperature at which winter wheat is sprayed in the spring need not be high, which is seldom the case in the field at that particular time of the year where wheat is commonly grown.

Table 22 shows that the optimum metal ion concentration in formulations used for wheat in these examples is about 15 mM, being superior to the formulation containing 20 mM in metal ions by about 47%. The prior art teaches that the preferred metal ion concentration used in triacontanol formulations about 20 to 25 mM or more. An interesting result is noted when wheat seedlings are subjected to water stress, such as drought conditions each day between spraying the seedlings and harvest, i.e., the water content of the seedlings rises very sharply, somewhat at the expense of dry weight increases. This may indicate that the formulations of the invention are useful in induction of drought resistance in



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plant life, and while drought appears to influence the dry weight increases observed to some degree, as would be expected, they are still observed, and statistically significant. Normal increases in water content observed in the case of wheat, and many other plants tested, is on the order of between about 7 to 10%, as compared to the 19 to 45% increases observed over control plots under the conditions of water stress described.

Concentrations of metal ions above about 20 mM in combination with the metal ions in the compositions of the present invention show a decrease in the results obtained, which amounts to only about 4% increase in dry weight over controls at a  $Ca^{+2}$  concentration of 25 mM.

Also, an increase in the dry weight of wheat seedlings was observed using a soil drench composition, shown in Table 22, which was 7% higher that the dry weight of controls treated with an identical solution containing Ca<sup>+2</sup> only. While this increase is only about one-half that observed in the case of foliar application of the formulations, the result may be expected to vary with the quantity of solution applied to the soil. Due to the larger quantity of solution required using soil drenches, however, this mode of application is not preferred, but nevertheless effective.

Results in field trials may be expected to equal or exceed those observed in these short-term greenhouse trials, as described in the foregoing Specification.



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1 EXAMPLE XVI: SWEET CORN

The formulations of the present invention were found active in promoting the growth of sweet corn seedlings through application in accordance with the methods of the invention.

5 The increases observed were found to about equal to those observed for field corn seedlings in greenhouse studies under the conditions used, however, the concentrations of metal ions of the invention required for a response was found to be somewhat higher than those used in the case of field corn.

10 Concentrations of metal ions in the formulations applied which may be considered optimum are between about 3 mM and 10 mM, with between about 7.5 mM and 10 mM being preferred for sweet corn (Table 26).

About two weeks prior to harvest, sweet corn plants in field trials showed about 22% more ears per plot that control plants on the average, with an increase in ear size also being apparent. Plants sprayed at the fourth-leaf stage produced marketable increases in yields up to 57% (see Table 27) for cv. Golden Cross Bantam using formulations containing 10 mM Ca<sup>+2</sup> and compounds of the invention.

## EXAMPLE XVII: TOBACCO

Tobacco seedlings were tested with formulations of the invention, with results shown in Table 28. Optimum concentrations of polyvalent metal ions useful on tobacco seedlings are of a broad range, with similar results being observed four days after foliar spraying with formulations containing between 5 mM and 15 mM Ca<sup>+2</sup>. Metal ions outside this range also produce effective plant growth stimulation. Similar activity is noted with other tobacco cultivars, with the preferred stage of application being about one to three weeks after seedlings have been set in the field. Application of the formulations of the present invention is best accomplished at temperatures of about 22° C or more, preferably between about 22° and 33° C, however, positive results may be observed



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outside this temperature range. Furthermore, tobacco seeds, which take a considerable amount of time to germinate and attain a size suitable for transplanting in the field, may be soaked in formulations of the present invention prior to planting in beds to aid in improving both germination and increasing the growth rate thereof.

## EXAMPLE XVIII: COTTON

Cotton seedlings respond favorably to the formulations described in the present invention, with increases in growth found in greenhouse trials shown in Table 29. The leaves of cotton seedlings are easily coated with aqueous solutions of the compositions of the invention without the aid of any surfactant additive, as are leaves of other dicots such as cowpeas and the like, when applied as foliar sprays, and improved responses are noted at temperatures preferably of 24°C and higher. Both the carboxylic acids and esters thereof of the invention (or their salts), and particularly waxy esters cause significant growth increases measured in terms of dry weights and compared to control plots. However, the inclusion of esters, and particularly waxy esters, show slight differences in the responses observed using carboxylic acids and their salts. Increases in the heights of the seedlings were also noted, however, these were found to be about 5 to 15% in difference as compared to controls.

# EXAMPLE XIX: PEPPERS

Pepper seedlings respond well to the various formulations of the present invention, with greenhouse trial results on cv. Early California Wonder being reported in Table 30. An unusual response to formulations containing different metal ion concentrations and different compounds of the invention is observed, with a somewhat different metal ion concentration being preferred for compounds of the invention having varying hydrocarbon chain lengths. Thus, while significant increases



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in dry weight were observed with seedlings sprayed with formulations containing triacontanoic acid and Ca<sup>+2</sup> (10 mM) or with tetracosanoic acid and Ca<sup>+2</sup> (5 mM), no significant increase was observed when the metal ion concentration was raised to 10 mM or more using the formulation containing tetracosanoic acid on this cultivar. At midseason, a large increase in the number of peppers on treated plants compared to the number of peppers on control plants was observed, and the final yield of peppers was found to be 82% over controls (see Tables and 36).

#### EXAMPLE XX: LETTUCE

Lettuce (cv. Buttercrunch) was treated with two formulations of the invention and allowed to mature prior to harvest. Table 32 shows the increases in marketable yield found using formulations of the invention containing beeswax and varying concentrations of Ca<sup>+2</sup>. Lettuce appears to respond to metal ion concentrations over a broad range as in the case of tobacco, reported hereinabove.

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## EXAMPLE XXI: POTATOES -

Table 33 shows that the formulations of the present invention are effective in stimulating the growth of potatoes. Also indicated by the data therein contained is that potatoes respond at a preferred temperature at the time of application below about 24° C, with a temperature of about 21° C being more preferred. This finding is advantageous to the grower since potatoes are genreally grown in cool climates, and a relatively higher temperature at the time the formulations of the invention may be applied is less likely to occur at the time of application of the formulations. It is important to note that root crops which show an increase in the dry weight of foliage obtained therefrom, also show similar increases in the size and weight of the crop itself when treated with the formulations of the present invention. For this reason,



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the dry weight of foliage from potato seedlings was used as an indication of growth to predict optimum formulations of the present invention useful in the field to produce increases in crop yield.

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#### EXAMPLE XXII: RADISHES

Table 34 shows the increases in the marketable yield of radishes found as a result of application of the compositions of the invention. It is apparent from inspection of the data that radishes respond preferably to those compounds of the invention of relatively low molecular weight. Thus, increases observed with the formulation containing beeswax (a mixture containing compounds of the formula R-COOR', wherein R and R' are alkyl groups of between 24 and 36 carbon atoms in length) and metal ions was found to be 35% over controls, while application of tetracosanoic acid (C24) alone showed an increase in yield of 47% over controls. High metal ion concentrations, i.e., 15 mM or more, are found to produce no significant increases in crop yield over controls under the conditions herein presented.

## EXAMPLE XXIII: GRASSES

Grasses of economic value, such as alfalfa, bluegrass, and the like, respond to formulations of the invention as evidenced by increases in the dry weight of the harvested grasses compared to controls (Tables 35, 37, and 38). Some grasses exhibit a growth-stimulating response at a narrow range of growth stages, such as Kentucky bluegrass (Table 35), while alfalfa generally responds over a somewhat broader range of stages of development (e.g., 8 to 20 cm in height, Tables 37 and 38). Grasses harvested for use as forage and the like generally respond to applications of formulations of the invention before the first cutting, and increases are observed with a second harvest without further application of the formulations.



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## EXAMPLE XXIV: VEGETABLES AND OTHER CROPS

Other crops tested also have shown significant increases in marketable yields over controls as a result of application of the formulations of the present invention. Beans and cucumbers show high increases in crop yield, and observations of other crops tested indicate that similar results are possible for all crops tested with the compositions of the invention (Table 39). Onions have shown an increase in size up to about 42% when treated with the formulations, and carrots have shown increases of about 36%. While all data contained herein was generated through the use of the compositions herein contained in the eastern United States, data generated under varying conditions in other portions of the U.S. indicate that similar results are possible under a wide range of environmental factors.

From the above description and examples of the invention, it becomes apparent to one skilled in the art that the formulations of the invention have a wide range of potential and applicability for the growth promotion of plant life and increase of the yields of crops. Polybasic acids are as effective as monobasic acids in the formulations (Table 3), and esters, especially waxy esters such as those contained in a wide variety of naturally-occurring waxes and oils and the like are very useful in the formulations of the present invention, and show some superiority in a number of cases when included in the compositions herein described.

As a result of the remarkable effectiveness of the compositions of the instant invention, other effects on plant are possible through the use thereof, such as improved disease resistance and cold resistance of plant life, and effects of this type have been observed. For example, sweet which was found susceptible to disease and produced no marketable yield on control plots was found to produce normal yields when treated previously with the formulations of the



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invention, and other effects similar thereto are also observable through the application of the formulations of the invention.

While not intending to be bound to any precise mechanism whereby the invention achieves its remarkable results, it is possible that the long-chain compounds of the invention may act as "coating agents" in minute quantities, which may subsequently aid plant life in the use of absorption of the metal ions of the invention as micronutrients, foliar fertilizer agents, and the like. While other mechanisms are possible, any precise mechanism remains unclear.

The impact of the use of the formulations of the present invention has a high potential in the increase of world food supply, as well as the economical increase of resources derived from plant life useful as energy sources, building materials, and the like. Many other uses, yet unrealized, may evolve from the use of plant life if the supply of plant life resources were to become economically plentiful, said uses being possible through the use of the compositions disclosed in the present invention. Application of the formulations is conveniently made by the methods herein described, and may be made by other methods practiced in the art, such as addition of the components of the formulations to irrigation systems, application of said components to plant life as side dressings to be further diluted with water and absorbed by the root systems of plant life, and the like.



The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims:



I CLAIM:

A plant growth stimulator composition,
 comprising: a compound of the formula:

R-COOR'

(I)

or a salt thereof wherein R is a saturated long-chain alkyl group having 15 to 47 carbon atoms, an unsaturated long-chain alkyl group having 15 to 47 carbon atoms, a saturated long-chain alkyl group having 15 to 47 carbon atoms which is substituted with 1 to 4 carboxyl groups or an unsaturated long-chain alkyl group having 15 to 47 carbon atoms which is substituted with 1 to 4 carboxyl groups; R' is hydrogen, a saturated alkyl group having 1 to 36 carbon atoms, an unsaturated alkyl group having 1 to 36 carbon atoms, a saturated alkyl group having 1 to 36 carbon atoms substituted with 1 to 4 carboxyl groups or an unsaturated alkyl group having 1 to 36 carbon atoms substituted with 1 to 4 carboxyl groups; and a metal salt wherein the metal ion of said salt has a valence of +2 to +3 in an aqueous solution, said metal salt being present in said formulation in an amount effective to assist said compound in stimulating plant growth. 20

- 2. The composition according to claim 1, wherein said formulation is a dry formulation comprising said compound and said metal salt.
- The composition according to claim 1, wherein
   said formulation is in the form of a liquid.
  - 4. The composition according to claim 1, wherein said formulation is in the form of an aqueous solution or dispersion and wherein said metal salts form metal ions in solution.



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- 5. The composition according to claim 2, wherein 2 said compound is coated onto particles of said salt.
- 6. The composition according to claim 1, wherein said compound is a compound which is found in a naturally3 occurring wax or oil.
  - 7. The composition according to claim 1, wherein said composition includes a naturally-occurring wax or oil which contains said compound.
    - 8. The composition according to claim 7, wherein said naturally-occurring wax or oil is selected from the group consisting of beeswax, esparto wax, peanut oil, jojoba oil, olive oil, soybean oil and rice bean oil.
- 9. The composition according to claim 7, wherein said composition contains beeswax.
  - 10. The composition according to claim 1, wherein said compound is a compound of the formula:

or a salt thereof wherein R is a saturated long-chain alkyl group having 15 to 47 carbon atoms and R' is hydrogen or a saturated alkyl group having between 1 to 36 carbon atoms.

- 11. The composition according to claim 10, wherein 2 R' is hydrogen.
- 12. The composition according to claim 10, wherein R' is a saturated long-chain alkyl group having between 3 1 to 36 carbon atoms.



- 13. The composition according to claim 4, wherein said metal ions are selected from the group consisting of Ca<sup>+2</sup>, La<sup>+3</sup>, Mg<sup>+2</sup>, Mn<sup>+2</sup>, Sr<sup>+2</sup>, Pb<sup>+2</sup>, Ba<sup>+2</sup>, Cd<sup>+2</sup>, Zn<sup>+2</sup>, 4 Cu<sup>+2</sup>, and Co<sup>+2</sup>.
- 14. The composition according to claim 4, wherein 2 said metal ion is  $Ca^{+2}$ .
- 15. The composition according to claim 1, wherein 2 said metal salt is a calcium salt.
- 16. The composition according to claim 7, wherein 2 said metal salt is a calcium salt.
- 17. The composition according to claim 1, wherein the weight ratio of said compound to said metal salt 3 is in the range of 1:5 to 1:500,000,000.
- 18. The composition according to claim 1, wherein said compound of the formula (I) is present in an amount of 50µg to 5g per kilogram of the composition.
  - 19. A method for stimulating plant growth which comprises applying an effective plant growth-stimulating amount of the composition according to claim 1, to the area where plants are growing.
  - 20. A method for stimulating plant growth which comprises applying an effective plant growth-stimulating amount of the composition of claim 3, to the area where 4 plants are growing.
  - 21. A method for stimulating plant growth which comprises applying an effective plant growth-stimulating amount of the composition of claim 4, to the area where 4 plants are growing.
    - 22. A method for stimulating plant growth which



comprises applying an effective plant growth-stimulating amount of the composition of claim 6, to the area where plants are growing.

- 23. A method for stimulating plant growth which comprises applying an effective plant growth-stimulating amount of the composition of claim 8, to the area where plants are growing.
- 24. A method for stimulating plant growth which comprises applying an effective plant growth-stimulating amount of the composition of claim 9, to the area where plants are growing.
- 25. A method for stimulating plant growth which comprises applying an effective plant growth-stimulating amount of the composition of claim 10, to the area where plants are growing.
- 26. A method for stimulating plant growth which comprises applying an effective plant growth-stimulating amount of the composition of claim 14, to the area where plants are growing.
- 27. A method according to claim 21, wherein said composition is applied to the leaves of growing plants.
- 28. A method according to claim 21, wherein said composition is applied when said plant life has between 2 and 7 true leaves.
  - 29. A method according to claim 19, wherein said plant life is selected from the group consisting of field corn, soybeans, peas, tomatoes, wheat, sweet corn, tobacco, cotton, peppers, lettuce, potatoes, radishes, carrots, alfalfa, barley, beans, sorghum and cucumbers.



- 30. A method for stimulating plant growth which comprises applying an effective amount of the composition according to claim 1, to plant life selected from the group consisting of field corn, soybeans, peas, tomatoes, wheat, sweet corn, tobacco, peppers, cotton, lettuce, potatoes, radishes, carrots, alfalfa, barley, beans, sorghum, cucumbers, bluegrass, onions and cowpeas.
  - 31. A plant growth stimulator composition, comprising:
  - $0.1 \mu g$  to 10 grams per kilogram of the composition of a compound of the formula:

or a salt thereof wherein R is a saturated long-chain alkyl group having 15 to 47 carbon atoms, an unsaturated long-chain alkyl group having 15 to 47 carbon atoms, a saturated long-chain alkyl group having 15 to 47 carbon atoms which is substituted with 1 to 4 carboxyl groups or an unsaturated long-chain alkyl group having 15 to 47 carbon atoms which is substituted with 1 to 4 carboxyl groups; R' is hydrogen, a saturated alkyl group having 1 to 36 carbon atoms, an unsaturated alkyl group having 1 to 36 carbon atoms, a saturated alkyl group having 1 to 36 carbon atoms substituted with 1 to 4 carboxyl groups or an unsaturated alkyl group having 1 to 36 carbon atoms substituted with 1 to 4 carboxyl groups or an unsaturated alkyl group having 1 to 36 carbon atoms

substituted with 1 to 4 carboxyl groups; and
50 grams to 1000 grams per kilogram of the composition
20 of a metal salt wherein the metal ion of said salt has
a valence of +2 or +3 in an aqueous solution.

32. The composition according to claim 31, wherein said compound of the formula (I) is present in an amount of 50µg to 5g per kilogram of the composition and said metal salt is present in an amount of 250g to 1000g 5 per kilogram of the composition.

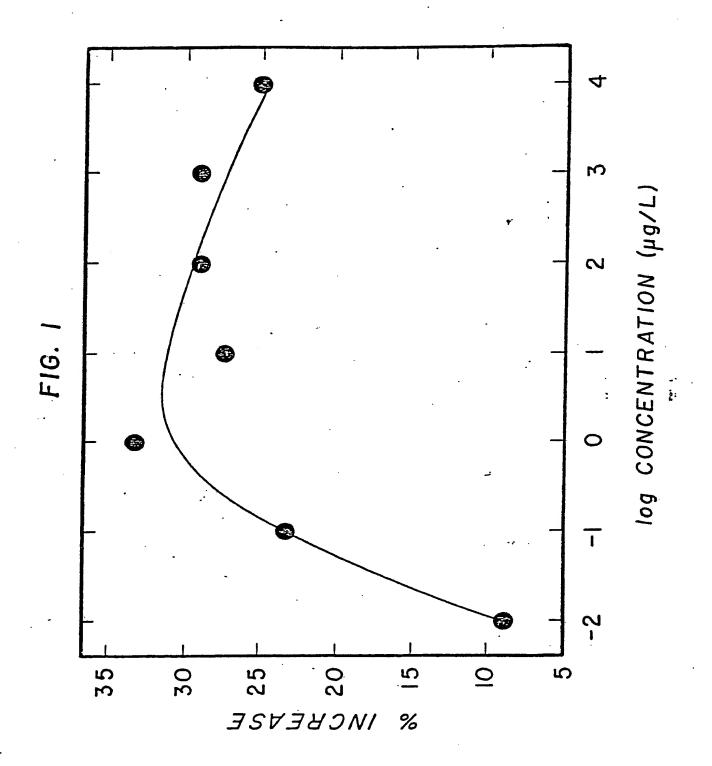


- 33. The composition according to claim 31, wherein said compound of the formula (I) is a compound which is found in a naturally-occurring wax or oil.
- 34. The composition according to claim 32, wherein said composition includes a naturally-occurring wax or oil selected from the group consisting of beeswax, esparto wax, peanut oil, jojoba oil, olive oil, soybean oil and rice bean oil.
- 35. The composition according to claim 32, wherein said composition contains beeswax.
  - 36. The composition according to claim 31, wherein said compound is a compound of the formula:

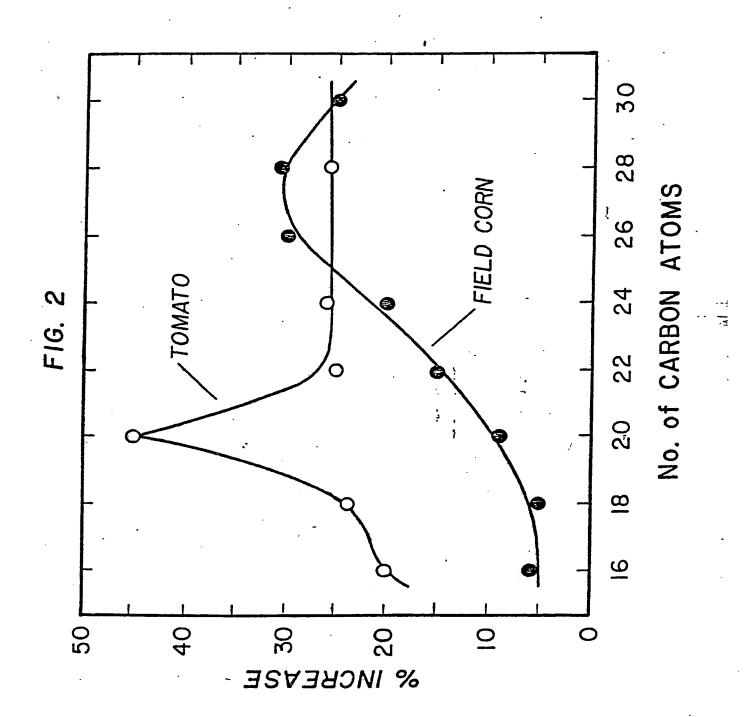
R-COOR' (I)

- or a salt thereof wherein R is a saturated long-chain alkyl group having 15 to 47 carbon atoms and R' is hydrogen or a saturated alkyl group having between 1 to 36 carbon atoms.
- 37. The composition according to claim 31, wherein 2 said metal salt is a calcium salt.
- 38. The composition according to claim 34, wherein 2 said metal salt is a calcium salt.
- 39. The composition according to claim 35, wherein 2 said metal salt is calcium chloride.
  - 40. The composition according to claim 31, wherein said metal ions are selected from the group consisting of  $Ca^{+2}$ ,  $La^{+3}$ ,  $Mg^{+2}$ ,  $Sr^{+2}$ ,  $Pb^{+2}$ ,  $Ba^{+2}$ ,  $Cd^{+2}$ ,  $Zn^{+2}$ ,  $Cu^{+2}$  and  $Co^{+2}$ .
- 41. A method for stimulating plant growth which comprises applying an aqueous solution containing an effective plant growth stimulating amount of the 4 composition of claim 31 to the leaves of growint plants

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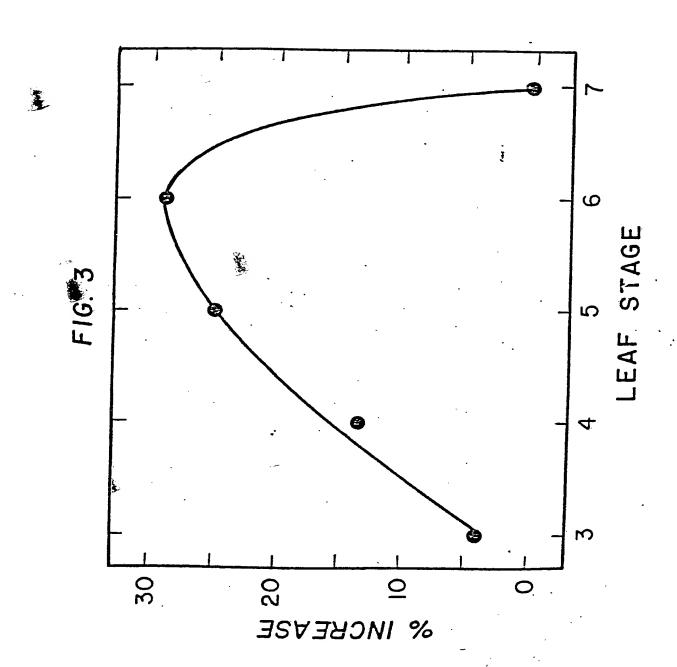




# INTERNATIONAL SEARCH REPORT

International Application No PCT/US83/00288								
1. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) 8								
According to International Patent Classification (IPC) or to both National Classification and IPC Intl. C13 A01N 59/00								
US C1. 71/84								
II. FIELDS SEARCHED								
Minimum Documentation Searched 4								
Classification System   Classification Symbols								
US	US 71/65,77,84,97,106,113							
Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched •								
Chemical Abstracts - Vol. 30-Vol. 97								
1. Calciumchoride 2. Beeswax, Soybean and Linseed Oil 3.								
All acids and esters specifically exemplified								
		ONSIDERED TO BE RELEVAN						
Category *	- <del>:</del>			Claim No. 18				
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	-	of cited documents: 15	"T" later document published after the internationa or priority date and not in conflict with the app	ilication out				
con	sidered to be	g the general state of the art which of particular relevance	cited to understand the principle or theory uninvention	serlying the				
"E" earlier document but published on or after the international filling date "X" document of particular relevance; the claimed invention cannot be considered to								
		may throw doubts on priority claim establish the publication date of a	s) or involve an inventive step					
citat	ion or other	special reason (as specified) ng to an oral disclosure, use, exhibi	cannot be considered to involve an inventive ste	such docu-				
othe	r means		ments, such combination being obvious to a pe	rson skilled				
"P" document published prior to the international filing date but in the art. later then the priority date claimed "&" document member of the same patent family								
IV. CERTIFICATION								
Date of the Actual Completion of the International Search   Date of Mailing of this International Search Report*								
June 17, 1983								
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ISA/US			Catherine Mills					

Form PCT/ISA/210 (second sheet) (October 1981)





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